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(21) International Application Number: PCT/US90/05294 (22) International Filing Date: 18 September 1990 (18.09.90) (30) Priority data: 410,647 21 September 1989 (21.09.89) US (71) Applicant: MICRO VESICULAR SYSTEMS, INC. [US/US]; 20 Cotton Road, Nashua, NH 03063 (US). (72) Inventor: WALLACH, Donald, F., H. ; 94 Dow Road, Hollis, NH 03049 (US). (74) Agents: LOREN, Ralph, A. et al.; Lahive & Cockfield, 60 State Street, Boston, MA 02109 (US).		(81) Designated States: AT (European patent), AU, BE (European patent), BF (OAPI patent), BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH (European patent), CM (OAPI patent), DE (European patent)*, DK (European patent), ES (European patent), FI, FR (European patent), GA (OAPI patent), GB (European patent), HU, IT (European patent), JP, LU (European patent), ML (OAPI patent), MR (OAPI patent), NL (European patent), NO, SE (European patent), SN (OAPI patent), SU, TD (OAPI patent), TG (OAPI patent). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: HYBRID PAUCILAMELLAR LIPID VESICLES (57) Abstract <p>Disclosed are hybrid paucilamellar lipid vesicles containing a phospho- or glycolipid and a nonionic, anionic or zwitterionic surfactant in the lipid bilayers. The paucilamellar vesicles may have either an aqueous or oil-filled central cavity. A method of manufacture for these vesicles is also disclosed. The paucilamellar lipid vesicles solve certain problems of cross-membrane transport, stability and cost, and may be used for transport of materials across membranes or skin, for diagnostic testing, or as markers or labels for visualization.</p>		

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HYBRID PAUCILAMELLAR LIPID VESICLES

Reference to Related Applications

This application is a continuation-in-part
5 of United States Patent Application Serial No.
157,571, filed March 3, 1988, entitled "Paucilamellar
Lipid Vesicles," which was a continuation-in-part of
United States Serial No. 025,525, filed March 13,
1987, entitled "Method of Producing High Aqueous
10 Volume Multilamellar Vesicles," now abandoned; United
States Patent Application Serial No. 078,658, filed
July 28, 1987, also entitled "Method of Producing
High Aqueous Volume Multilamellar Vesicles," now
United States Patent No. 4,855,090, issued August 8,
15 1989; and United States Patent Application Serial No.
124,824, filed November 25, 1987, entitled "Lipid
Vesicles Formed of Surfactants and Steroids."

Background of the Invention

The present invention relates to the
20 production of hybrid paucilamellar lipid vesicles.
More particularly, the present invention concerns
lipid vesicles which have phospholipids or
glycolipids in addition to single-chain non-ionic,
anionic, or zwitterionic surfactants as the major
25 components of the walls (or lipid bilayers) of a
paucilamellar lipid vesicle.

Lipid vesicles are substantially spherical
structures made of materials having a high lipid
content, e.g., surfactants or phospholipids. The

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lipids of these spherical vesicles are organized in the form of lipid bilayers. The lipid bilayers encapsulate an aqueous volume which is either interspersed between multiple onion-like shells of lipid bilayers (forming multilamellar lipid vesicles or "MLV") or the aqueous volume is contained within an amorphous central cavity. The most commonly known lipid vesicles having an amorphous central cavity filled with aqueous medium are the unilamellar lipid vesicles. Large unilamellar vesicles ("LUV") generally have a diameter greater than about 1 μ while small unilamellar lipid vesicles ("SUV") generally have a diameter of less than 0.2 μ . Lipid vesicles have a variety of uses including adjuvants or carriers for a broad spectrum of materials.

Although substantially all the investigation of lipid vesicles in recent years has centered on multilamellar and the two types of unilamellar lipid vesicles, a fourth type of lipid vesicle, the paucilamellar lipid vesicle ("PLV"), exists. See Callo and McGrath, Cryobiology 1985, 22(3), pp. 251-267. This lipid vesicle has barely been studied until recently and had only been manufactured with phospholipids surrounding an amorphous aqueous-filled volume. PLV's consist of about 2 to 10 peripheral bilayers surrounding a large, unstructured central cavity. In all PLV's described previous to United States Patent Application Serial No. 157,571, the disclosure of which is incorporated herein by reference, this central cavity was filled with an aqueous solution. The cited application first disclosed oil-filled vesicles.

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Each type of lipid vesicle appears to have certain uses for which it is best adapted. For example, MLV's have a higher lipid content than any of the other lipid vesicles, so to the extent that a lipid vesicle can encapsulate or carry a lipophilic material in the bilayers without degradation, MLV's have been deemed the most advantageous for carrying lipophilic materials. In contrast, the amount of water encapsulated in the aqueous shells between the lipid bilayers of the MLV's is much smaller than the water which can be encapsulated in the central cavity of LUV's, so LUV's have been considered advantageous in transport of aqueous material. However, LUV's, because of their single lipid bilayer structure, are not as physically durable as MLV's and are more subject to enzymatic degradation. SUV's have neither the lipid or aqueous volumes of the MLV's or LUV's but because of their small size have easiest access to cells in tissues.

PLV's, which can be considered a sub-class of the MLV's, possess features of both MLV's and LUV's. PLV's appear to have advantages as transport vehicles for many uses as compared with the other types of lipid vesicles. In particular, because of the large unstructured central cavity, PLV's are easily adaptable for transport of large quantities of aqueous- or oil-based materials. Moreover, the multiple lipid bilayers of the PLV's provides PLV's with additional physical strength and resistance to degradation as compared with the single lipid bilayer of the LUV's. As illustrated in the present application and the previously cited United States

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Patent Application Serial No. 157,571, the central cavity of the PLV's can be filled wholly or in part with an apolar oil or wax and then can be used as a vehicle for the transport or storage of hydrophobic materials. The amount of hydrophobic material which can be transported by the PLV's with an apolar core is much greater than can be transported by MLV's.

Conventional methods for producing multilamellar lipid vesicle start by dissolving the lipids, together with any lipophilic additives, in an organic solvent. The organic solvent is then removed by evaporation using heat or by passing a stream of an inert gas (e.g., nitrogen) over the dissolved lipids. The residue is then hydrated with an aqueous phase, generally containing electrolytes and additives such as hydrophilic biologically-active materials, to form multilamellar lipid membrane structures. In some variations, different types of particulate matter or structures have been used during the evaporation process to assist in the formation of the lipid residue. Changing the physical structure of the lipid residue can result in formation of better vesicles upon hydration. Two recent review publications, Gregoriadis, G., ed. Liposome Technology (CRC, Boca Raton, Fl.), Vols. 1-3 (1984), and Dousset and Douste-Blazy (in Les Liposomes, Puisieux and Delattre, Editors, Techniques et Documentation Lavoisier, Paris, pp.41-73 (1985)), summarize the methods which have been used to make MLV's.

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No matter how the MLV's or PLV's are formed, once made it is necessary to determine the effectiveness of the process. Two measurements commonly used to determine the effectiveness of encapsulation of materials in lipid vesicles are the encapsulated mass and captured volume. The encapsulated mass is the mass of the substance encapsulated per unit mass of the lipid and is often given as a percentage. The captured volume is defined as the amount of the aqueous phase trapped inside the vesicle divided by the amount of lipid in the vesicle structure, normally given in ml liquid/g lipid.

Phospholipid vesicles, while mimicking membrane structure because of similarity of materials with naturally occurring membranes, have a number of problems. First, isolated phospholipids are subject to degradation by a large variety of enzymes. Second, the most easily available phospholipids are those from natural sources, e.g., egg yolk lecithin, which contain polyunsaturated acyl chains that are subject to autocatalyzed peroxidation. When peroxidation occurs, the lipid structure breaks down, causing fracture of the lipid vesicle and premature release of any encapsulated material. While hydrogenation may be used to saturate the chains, it is an expensive process which raises the already high cost of the phospholipid starting materials, as well as changing the vesicle stability.

Because of these problems with using plain phospholipids, certain companies, primarily L'Oreal

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and Micro Vesicular Systems, have been using non-ionic surfactants to form the structure of vesicles. L'Oreal uses primarily polyglycols, e.g., see United States Patents Serial Nos. 4,772,471 and 4,217,344, while Micro Vesicular Systems has been using primarily polyoxyethylene fatty acid ethers and esters (see United States Patent Application Serial No. 157,571 and United States Patent No. 4,855,090). The L'Oreal vesicles appear to be classic MLV's while the Micro Vesicular Systems vesicles are primarily PLV's.

For certain uses, e.g., transportation of vesicles through membranes or permeation of the skin, the presence of a small amount of phospholipid and/or glycolipid to the bilayer structure of the vesicles may be important. A problem with using the phospholipids or glycolipids in conjunction with many synthetic surfactants is that most of the surfactants have a non-ionic head group linked to a single hydrophobic chain while most phospholipids and glycolipids have two hydrophobic chains linked to an ionic head group. Use of both single and multiple chain molecules in the structure of vesicle walls may lead to problems in the packing of the lipids which form the lipid bilayers. Under most circumstances, one would expect that any attempt to form a stable vesicle by blending single and multiple chain lipids, particularly when one is a non-ionic lipid while the other is an ionic or zwitterionic lipid, would be difficult at best. In addition, the phospholipids are still subject to phospholipases after vesicle formation. However, the hybrid vesicles are exactly

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what is needed to solve certain problems of cross-membrane transport, stability and cost.

Accordingly, an object of the invention is to provide stable hybrid lipid vesicles having a
5 non-ionic, zwitterionic, or anionic surfactant and a phospholipid or glycolipid in the lipid bilayers of the vesicles.

A further object of the invention is to provide stable hybrid paucilamellar lipid vesicles
10 encapsulating a water-immiscible material within the central amorphous cavities of the vesicles.

Another object of the invention is to provide a method of manufacture of hybrid vesicles.

A still further object of the invention is
15 to provide a vehicle for the transport of oil-soluble or water-soluble materials into the skin.

These and other objects and features of the invention will be apparent from the following description.

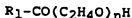
20 Summary of the Invention

The present invention features hybrid paucilamellar lipid vesicles having phospholipids or glycolipids in addition to single-chain non-ionic, anionic, or zwitterionic non-phospholipids in the
25 lipid bilayers. These hybrid vesicles are

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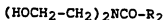
particularly useful for transport of oil-soluble or water-soluble material into the skin.

The hybrid paucilamellar lipid vesicles having phospholipids or glycolipids in addition to non-ionic or zwitterionic surfactants in their lipid bilayers consist of 2-10 lipid bilayers arranged in the form of substantially spherical shells separated by aqueous layers surrounding a large amorphous central cavity free of lipid bilayers. The lipid bilayers have about 0-30% phospholipids and/or glycolipid and 0-75% single-chain non-ionic, anionic, or zwitterionic surfactant, preferably with other materials such as 0-25% of a sterol and 0-5% of a charge-producing agent. The preferred non-ionic surfactants are selected from the group consisting of polyoxyethylene fatty ethers having the formula



where R_1 is lauric, myristic, or palmitic acid or their derivatives, single or double unsaturated octadecyl acids or their derivatives, or double unsaturated eicodienoic acids or their derivatives and n ranges from 2-4;

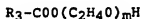
diethanolamides having the formula



where R_2 is caprylic, lauric, myristic, palmitic, stearic, or linoleic acid or their derivatives;

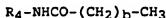
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polyoxyethylene fatty acid esters having the
formula



where R_3 is lauric, myristic, palmitic,
5 stearic, or oleic acids or their derivatives, double
unsaturated octadecyl acids or their derivatives, or
double unsaturated eicodienoic acids or their
derivatives and m ranges from 2-4;

long chain acyl hexosamides having the
10 formula



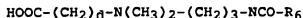
where b ranges from 10-18 and R_4 is a sugar
molecule selected from a group consisting of
glucosamine, galactosamine, and N-methylglucamine;

15 long chain acyl amino acid amides having the
formula



where c ranges from 10-18 and R_5 is an amino
acid side chain;

20 long chain acyl amides having the formula



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where R_6 is an acyl chain having 12-20 carbons and not more than two unsaturations, and d ranges from 1-3;

polyoxyethylene (20) sorbitan mono- or trioleate;

5 polyoxyethylene glyceryl monostearate with 1-10 polyoxyethylene groups;

glycerol monostearate;

sarcosinamides having the formula

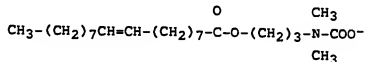


where R_7 is selected from the group consisting of single-chain carbonyl derivatives of C_{12} - C_{20} fatty acids; and

betaines having the formula



where R_8 is selected from the group consisting of long-chain fatty acid esters, most preferably oleoyl propyl betaine having the formula



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Phospholipids and/or glycolipids particularly useful in the invention include phosphatidylcholines, phosphatidylethanolamines, phosphatidylserines, inositolphosphatides, sphingomyelins, ceramides, cerebrosides, gangliosides, sulfatides, and mixtures and derivatives thereof. Phospholipids or glycolipids with saturated hydrocarbon chain length greater than sixteen are not as useful as smaller chain or unsaturated chain molecules in the invention because they lack some chain fluidity. If a charge-producing agent is used, preferred charge-producing agents include dicetyl phosphate, quaternary ammonium salts, cetyl sulfate, sarcosinamides, phosphatidic acid, phosphatidyl serine, and fatty acids such as oleic acid or palmitic acid.

The preferred hybrid paucilamellar vesicles of the invention have a central cavity carrying either water-soluble materials or water-immiscible oily-solution, preferably selected from a group consisting of oils, waxes, natural and synthetic triglycerides, acyl ethers, petroleum derivatives and their analogues and derivatives, encapsulated within the central amorphous cavity. This type of water-immiscible material can act as a carrier for materials which are not soluble in an aqueous phase. In addition, it may also be used for materials which are not dissolvable or soluble in the lipids which form the bilayers. If the water-immiscible oil, such as a peanut oil, tristearin or mineral oil is used, the amount of non-ionic or zwitterionic surfactant which is needed to form stable vesicles is

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decreased. In fact, under certain circumstances, oil-filled paucilamellar vesicles may be formed using the methods of the invention without the addition of any non-ionic or zwitterionic surfactant. However, the most stable hybrid vesicles appear to be formed with 10-30% phospholipid and/or glycolipid, 5-20% of a sterol which is cholesterol, about 1% of a charge-producing agent such as oleic acid, and the remainder constituting the surfactant.

10 The invention further features a method of producing the hybrid vesicles of the invention. The phospholipid and/or glycolipid and non-ionic or zwitterionic surfactant are blended, with heating if necessary, until a homogeneous lipid layer is
15 formed. If a water-immiscible oil is to be encapsulated, it is blended in the already formed lipid phase, forming a lipophilic phase. If any oil-soluble or oil-suspendable materials are to be encapsulated within the paucilamellar vesicles, they
20 are first dispersed in the oil. The term "dispersed" as used herein includes dissolution or forming a suspension or colloid to yield a flowable phase. If no oil is used, the lipid phase is the lipophilic phase.

25 Once a lipophilic phase is made, it is blended with an aqueous phase under shear mixing conditions to form the vesicles. "Shear mixing" is defined as the mixing of the lipophilic phase with the aqueous phase under turbulent or shear conditions
30 which provide adequate mixing to hydrate the lipid and form lipid vesicles. Shear mixing achieved by

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liquid shear which is substantially equivalent to a relative flow rate for the combined phases of about 5-30 m/s to a 1 mm orifice. The use of shear mixing conditions disrupts any lamellae which may form so
5 that the vesicles are formed without the formation of a separable lamellar phase.

The same materials which have been previously described for the vesicles are useful in the methods of the invention. Briefly, the formed
10 lipophilic phase is shear mixed with an excess of aqueous, e.g., 10:1:aqueous:lipid, and the resulting vesicles, which form in under a second, are then separated and can be used any of a variety of other uses.

15 The following description will further explain the invention.

Detailed Description of the Invention

The hybrid vesicles of the invention can be used for a variety of purposes, including the
20 function of a carrier for transport of materials across membranes or skin that would otherwise not be transportable. In fact, the vesicles of the invention could be used for any purpose where lipid vesicles such as liposomes are now being used or
25 contemplated. These lipid vesicles are characterized by 2-10 lipid bilayers or shells with small aqueous volumes separating each substantially spherical lipid shell. The innermost lipid bilayer surrounds a large, substantially amorphous central cavity which

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may be filled with either an aqueous solution or a water-immiscible oil. This central cavity acts as a "cargo hold," allowing delivery of a variety of materials to the desired location.

- 5 For certain uses, the incorporation of a charge producing amphiphile, yielding a net positive or negative charge to the lipid vesicles, is helpful. The preferred negative charge producing materials are carboxylic acids such as oleic and
- 10 palmitic acids, dicetyl phosphate, cetyl sulphate, sacrosinamides, phosphatidic acid, phosphatidyl serine, and mixtures thereof. In order to provide a net positive charge to the vesicles, long chain amines, e.g., stearyl amines or oleyl amines,
- 15 cationic local anaesthetics such as lidocaine, long chain pyridinium compounds, e.g., cetyl pyridinium chloride, quaternary ammonium compounds, or mixtures of these can be used.

- The vesicles may also include targeting
- 20 molecules, either hydrophilic or amphiphilic, which can be used to direct the vesicles to a particular target in order to allow release of the material encapsulated in the vesicle at a specified biological location. If hydrophilic targeting molecules are
- 25 used, they can be coupled directly or via a spacer to a residue of the polar portion of the surfactant, or they can be coupled, using state of the art procedures, to molecules such as palmitic acid, long chain amines, or phosphatidyl ethanolamine. If
- 30 spacers are used, the targeting molecules can be interdigitated into the hydrophilic core of the

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bilayer membrane via the acyl chains of these compounds. Preferred hydrophilic targeting molecules include monoclonal antibodies, other immunoglobulins, lectins, and peptide hormones.

5 For certain uses, targeting molecules which were linked to the lipid bilayers themselves, through the use of a sulfhydryl bond, are preferred. United States Patent Application Serial No. 320,944 describes a linkage of this type which yields high
10 efficiency targeting. Molecules containing sulfhydryl groups are incorporated into the structure of the bilayers and a bifunctional cross-linking reagent is used to link the targeting molecule to the bilayers.

15 In addition to hydrophilic targeting molecules, it is also possible to use amphiphilic targeting molecules. Amphiphilic targeting molecules are normally not chemically coupled to the surfactant molecules but rather interact with the lipophilic or
20 hydrophobic portions of the molecules constituting the bilayer lamellae of the lipid vesicles. Preferred amphiphilic targeting molecules are neutral glycolipids, galactocerebrosides (e.g., for hepatic galactosyl receptors), or charged glycolipids such as
25 gangliosides.

Vesicles made using the methods of the present invention can be used in diagnostic testing, e.g., agglutination testing of immunological systems. The vesicles can also be used as markers or
30 labels for visualization, e.g., swelling or shrinking

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in the presence of an immune reaction, or for radiography or NMR.

Hydrophilic materials which can be encapsulated include minerals such as titanium dioxide and silicas, viruses, macromolecules, immunological adjuvants such as muramyl dipeptide, peptide hormones such as insulin, calcitonin and glucagon, hypothalamic peptides, pituitary hormones, growth factors such as angiogenic, epithelial and epidermal growth factors, lymphokines such as interleukin-2 and interferon, blood proteins such as hemoglobin and Factor VIII, water-soluble plant hormones and pesticides, radionucleotides, contrast materials for radiological and NMR diagnosis, cancer cytostatics, and antibiotics. Examples of lipophilic materials which can be encapsulated include steroid hormones, pheromones, porphyrins, organic pesticides, fungicides, insect repellents, lipophilic vitamins and derivatives, alkyds, epoxy polyurethanes, fluorocarbons, and related resins. Oil based materials include an exclusive listing of additional lipophilic materials and materials which form colloids or suspensions in oil. A more complete listing of the types of pharmaceuticals that could be encapsulated in lipid vesicles is included in Gregoriadis, G., ed. Liposome Technology (CRC, Boca Raton, Fl.), Vols. 1-3 (1984).

The paucilamellar lipid vesicles can be made by a variety of devices which provides sufficiently high shear for shear mixing. There are a large variety of these devices available on the market

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including a microfluidizer such as is made by Biotechnology Development Corporation, a "French"-type press, or some other device which provides a high enough shear force and the ability to
5 handle heated, semiviscous lipids. If a very high shear device is used, it may be possible to microemulsify powdered lipids, under pressure, at a temperature below their normal melting points and still form the lipid vesicles of the present
10 invention.

A device which is particularly useful for making the lipid vesicles of the present invention has been developed by Micro Vesicular Systems, Inc., Vineland, New Jersey and is further described in
15 United States Patent Application Serial No. 163,806, filed May 3, 1988. Briefly, this device has a substantially cylindrical mixing chamber with at least one tangentially located inlet orifice. One or more orifices lead to a reservoir for the lipophilic
20 phase, mixed with an oil phase if lipid-core PLV's are to be formed, and at least one of the other orifices is attached to a reservoir for the aqueous phase. The different phases are driven into the cylindrical chamber through pumps, e.g., positive
25 displacement pumps, and intersect in such a manner as to form a turbulent flow within the chamber. The paucilamellar lipid vesicles form rapidly, e.g., less than 1 second, and are removed from the chamber through an axially located discharge orifice. In a
30 preferred embodiment, there are four tangentially located inlet orifices and the lipid and aqueous phases are drawn from reservoirs, through positive

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displacement pumps, to alternating orifices. The fluid stream through the tangential orifices is guided in a spiral flow path from each inlet or injection orifice to the discharge orifice. The flow paths are controlled by the orientation or placement of the inlet or injection orifices so as to create a mixing zone by the intersection of the streams of liquid. The pump speeds, as well as the orifice and feed line diameters, are selected to achieve proper shear mixing for lipid vesicle formation. As noted, in most circumstances, turbulent flow is selected to provide adequate mixing.

For small scale, e.g., experimental uses, shear mixing may be carried out using merely a series of syringes and a stopcock joining them. This "syringe method" uses one syringe containing the lipophilic phase, heated if necessary for flowability, which is then linked, via a stopcock, to a second, larger syringe containing an excess of an aqueous phase. The lipid and aqueous phase are then blended rapidly through the stopcock for a short time, e.g., normally less than a minute. This blending causes sufficient shearing to form the paucilamellar vesicles of the invention without the formation of an intermediate or separate lamellar phase. In fact, the formation of a separable lamellar phase would so clog or disrupt the flow as to make this method impossible to use.

When the water-immiscible oil is used, the oil displaces a portion of the aqueous phase as the vesicles are formed. The oil stabilizes the

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vesicles, leading to high fracture strength and longer term stability than vesicles made without oil. Although it is not necessary for understanding the invention, it is theorized that a very small amount of the surfactant acts as a stabilizing agent, stabilizing the boundary between the aqueous volume and the oil volume, allowing the oil droplet to form. In fact, the oil-filled vesicles are so stable that paucilamellar oil-filled vesicles can be formed from phospholipids using the methods of the invention without the addition of any non-ionic or zwitterionic surfactant, while aqueous-filled vesicles cannot be formed using the same materials and methods.

The following Examples will more clearly illustrate and delineate the scope of the present invention.

Example 1.

In this invention, egg yolk phosphatidylcholine (Lipoid 100, Lipoid ViCt, Ludwigshafen, GFR), was used in conjunction with a polyoxyethylene ether surfactant to form stable aqueous-based vesicles. These vesicles show high lipid uptake per/g of lipid and small size.

Table I lists the ingredients used to make the vesicles of this Example.

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TABLE 1.

Egg Yolk Phosphatidylcholine	1.00 g
Polyoxyethylene-2 Cetyl Ether (Brij 52)	1.00 g
Cholesterol	0.25 g
5 Oleic Acid	0.02 g

Briefly, approximately 1 g of egg yolk phosphatidylcholine was blended with 1 g of polyoxyethylene-2 cetyl ether (Brij 52, ICI Americas, Inc.), 0.25 g cholesterol (Sigma Chemical Co.) and
10 0.02 g oleic acid (J. T. Baker) as a charge-producing agent at approximately 65°C. A homogeneous lipid phase was obtained. One ml of the lipid phase was then shear mixed with 9 ml of phosphate buffered saline for approximately 2 minutes using a syringe
15 method as described below.

The lipophilic phase is placed in a 10 ml syringe and is attached through a stopcock having about a 1 mm orifice to a 25 ml syringe which contains the aqueous phase, phosphate buffered
20 saline. After the injection of the lipid phase into the aqueous phase, a second 25 ml syringe replaces the 10 ml syringe and the solution is rapidly forced through the stopcock from one syringe to the other. The resulting vesicles form in less than 1 minute.

25 The milky suspension which was obtained by the syringe method was combined with 20% dextran in saline at a centrifuge of 3000 rpm's for 15 minutes in a Beckman GP centrifuge. A liposomal layer separated at the top of the centrifuge tube.

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Microscopic examination showed spherical paucilamellar lipid vesicles which were not distinguishable from vesicles which did not have the phosphatidylcholine. The mean particle diameter was approximately 0.171 μ and the volume uptake was approximately 7.2 ml of phosphate buffered saline/g lipid.

Example 2.

This Example illustrates the oil-based paucilamellar vesicles of the invention. A lipid phase identical to that described in Example 1 was manufactured. One ml of that lipid phase was then blended with 1 ml of mineral oil (Drakeol 19), forming a lipophilic phase. The resulting 2 ml of the lipophilic phase was then blended with 9 ml of phosphate buffered saline using the syringe technique as described above.

Again, a milky solution is obtained which upon dextran centrifugation yields oil-filled paucilamellar vesicles. All of the oil is encapsulated within the vesicles, which also have a volume uptake of approximately 3 ml of the phosphate buffered saline/g lipid (excluding the mineral oil). The mean particle diameter was approximately 0.654 μ .

Example 3.

This Example illustrates that the methods of the invention may be used to form phospholipid

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paucilamellar oil-filled vesicles. Table 2 gives the ingredients used.

TABLE 2.

	Egg Yolk Phosphatidylcholine	1.00 g
5	Cholesterol	0.20 g
	Oleic Acid	0.02 g
	Mineral Oil (Drakeol 19)	1 ml

- One g of egg yolk phosphatidylcholine was blended with 0.2 g cholesterol and 0.02 g oleic acid by heating to 65°. This lipid phase was then blended with 1 g mineral oil (Drakeol 19) to form a lipophilic phase. Approximately 2 ml of lipophilic phase was then blended, using the syringe technique as previously described, with 4 ml of phosphate buffered saline. The resulting milky suspension was separated on a 20% dextran and saline gradient using centrifugation of 3,000 rpm for 15 minutes. A 3 ml liposomal layer separated on top of the centrifuge tube.
- 20 Microscopic examination showed spherical, oil-filled paucilamellar lipid vesicles. The aqueous volume uptake was approximately 3 ml/g phospholipid.

Example 4.

- This Example and the following Examples (Examples 5-7) illustrate the use of a mixed phospholipid/glycolipid as part of the structure of the paucilamellar vesicles of the invention. Table 3

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illustrates the materials used to form the lipid bilayers in this Example.

TABLE 3.

Type VIII Brain Extract	0.76 g
5 Polyoxyethylene-5 Oleyl Ether	1.99 g
Cholesterol	0.58 g
Oleic Acid	0.08 g

The type VIII brain extract, which is 30% sphingomyelin (a phospholipid), 30% cerebroside (a glycolipid), 10% sulfatide, and the balance other brain lipids is blended with polyoxyethylene-5 oleyl ether, cholesterol and oleic acid at approximately 65°C. to form a lipid phase. Approximately 1 ml of this lipid phase was mixed with 9 ml of phosphate buffered saline using the previously described syringe technique. Upon dextran centrifugation, spherical lipid vesicles encapsulating an aqueous phase could be seen under a microscope. The mean particle diameter was approximately 0.21 μ and the volume uptake was approximately 2.7 ml saline/g lipid.

Example 5.

In this Example, the mixed brain extract of Example 4 was used to make oil-centered vesicles. The lipid phase was made as described in Example 4 and 1 ml of the lipid phase was combined with an equal volume of peanut oil. The resulting mixture was made into vesicles using the same syringe technique described in Example 4. After dextran

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centrifugation, vesicles were separated which had a mean diameter of 1.66 microns and a volume uptake of 3.5 ml liquid/g lipid.

Surprisingly, use of mineral oil in place of
5 the peanut oil would not produce vesicles.

Example 6.

In this Example and the following Example, the same brain extract as was used in Examples 4 and 5 is used to make vesicles, except an entirely
10 different type of surfactant, diethanolamine linoleamide, was added.

Table 4 shows the lipids used in the manufacture of the lipid phase of this Example.

TABLE 4.

15	Type VIII Brain Extract	0.76 g
	Diethanolamine Linoleamide	1.78 g
	Cholesterol	0.85 g
	Oleic Acid	0.10 g

Approximately 0.76 g of the type VIII brain extract
20 was blended with 1.78 g of diethanolamine linoleamide, 0.85 g cholesterol and 0.10 g oleic acid at 65°C. One ml of this lipid phase was mixed, using the syringe technique, with 8 ml of phosphate buffered saline. After dextran centrifugation, lipid
25 vesicles were observed, showing a mean particle

- 25 -

diameter of approximately 0.263 μ . The volume uptake was 5 ml of saline/g lipid.

Example 7.

In this Example, the same lipid phase was
5 used as in Example 6 except oil-centered vesicles
were made. After the formation of the lipid phase,
1 ml of the lipid phase was blended with 1 ml peanut
oil before shear mixing using the syringe technique
with 8 ml of the phosphate buffered saline. Upon
10 separation, spherical vesicles with the mean particle
diameter of approximately 0.323 μ were observed. The
volume uptake was approximately 4.5 ml/g lipid.

As with Example 6, mineral oil would not
form vesicles.

15 As can be seen from the results of these
Examples, paucilamellar lipid vesicles having high
water or oil uptake can be formed with the materials
and methods of the present invention. Other testing
has shown that if different methods are used, e.g.,
20 the Bangham method for manufacture of lipid vesicles,
paucilamellar lipid vesicles are not formed using the
same materials but rather classic multilamellar lipid
vesicles are formed. These MLV's yield a much lower
water uptake as compared with PLV's and they exhibit
25 substantially no oil uptake.

The foregoing description is illustrative
only and those skilled in the art may find other

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materials and methods which accomplish the same results. Such other materials and methods are included within the following claims.

What is claimed is:

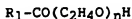
- 27 -

1. Hybrid paucilamellar lipid vesicles having a first lipid selected from a group consisting of phospholipids, glycolipids, and mixtures thereof, and a second lipid selected from the group consisting of non-ionic surfactants, zwitterionic surfactants, anionic surfactants, and mixtures thereof, as structural materials in lipid bilayers, said vesicle containing 2-10 lipid bilayers in the form of concentric substantially spherical shells separating aqueous layers, the innermost of said lipid bilayers enclosing a substantially amorphous central cavity, said vesicle bilayers comprising:

0-30% said first lipid; and

0-75% second lipid, said second lipid being selected from the group consisting of

polyoxyethylene fatty acid ethers having the formula



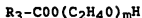
where R_1 is lauric, myristic, or palmitic acid or their derivatives, single or double unsaturated octadecyl acids or their derivatives, or double unsaturated eicodienoic acids or their derivatives, and n ranges from 2-10; and

diethanolamides having the formula



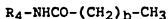
- 28 -

where R_2 is caprylic, lauric, myristic, stearic, or linoleic acid or their derivatives;



where R_3 is lauric, myristic, palmitic, stearic, or oleic acids or their derivatives, double unsaturated octadecyl acids or their derivatives, or double unsaturated eicodienoic acids or their derivatives and m ranges from 2-4;

long chain acyl hexosamides having the formula



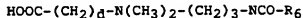
where b ranges from 10-18 and R_4 is a sugar molecule selected from a group consisting of glucosamine, galactosamine, and N-methylglucamine;

long chain acyl amino acid amides having the formula



where c ranges from 10-18 and R_5 is an amino acid side chain;

long chain acyl amides having the formula



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where R_6 is an acyl chain having 12-20 carbons and not more than two unsaturations, and d ranges from 1-3;

polyoxyethylene (20) sorbitan mono- or trioleate;

5 polyoxyethylene glyceryl monostearate with 1-10 polyoxyethylene groups;

glycerol monostearate;

sarcosinamides having the formula



where R_7 is selected from the group consisting of single-chain carbonyl derivatives of C_{12} - C_{20} fatty acids; and

betaines having the formula



where R_8 is selected from the group consisting of long-chain fatty acid esters.

20 2. The hybrid vesicles of claim 1 wherein said lipid bilayers further comprise a sterol or a derivative or chemical analogue thereof.

- 30 -

3. The hybrid vesicles of claim 2 wherein said sterol is selected from the group consisting of cholesterol, hydrocortisone, and derivatives and chemical analogues thereof.

5 4. The hybrid vesicles of claim 2 wherein said lipid bilayers further comprise a charge-producing agent.

5. The hybrid vesicles of claim 4 wherein said charge-producing agent is selected from the group
15 consisting of carboxylic acids, dicetyl phosphate, cetyl sulfate, phosphatidic acid, phosphatidyl serine, quaternary ammonium salts, cationic local anaesthetics, and mixtures and derivatives thereof.

6. The hybrid vesicles of claim 1 wherein said
15 first lipid is selected from the group consisting of phosphatidylcholines, phosphatidylethanolamines, phosphatidylserines, inositolphosphatides, sphingomyelins, ceramides, cerebroside, gangliosides, sulfatides, and derivatives and
20 chemical analogues thereof.

7. The hybrid vesicles of claim 1 wherein said vesicles comprises oil-filled paucilamellar lipid vesicles.

8. The hybrid vesicles of claim 7 wherein said
25 amorphous central cavity of said oil-filled paucilamellar vesicles comprises a water-immiscible oil.

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9. The hybrid vesicles of claim 8 wherein said water-immiscible oil is selected from the group consisting of oils, waxes, natural and synthetic triglycerides, acyl ethers, petroleum derivatives, and their derivatives and chemical analogues.

10. A method of making hybrid paucilamellar lipid vesicles comprising the steps of:

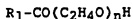
forming a lipid phase by blending a first lipid selected from the group consisting of phospholipids, glycolipids, and mixtures thereof, a second lipid selected from the group consisting of non-ionic surfactants, zwitterionic surfactants, anionic surfactants, and mixtures thereof, and any other lipophilic molecules to be incorporated into the lipid bilayers of said vesicles;

forming an aqueous phase by blending an aqueous-based solution and any aqueous soluble materials to be incorporated into said vesicles; and

shear mixing said lipid phase and said aqueous phase to form said hybrid vesicles without the formation of a separable lamellar phase.

11. The method of claim 10 wherein said second lipids are selected from the group consisting of

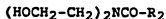
polyoxyethylene fatty acid ethers having the formula



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where R_1 is lauric, myristic, or palmitic acid or their derivatives, single or double unsaturated octadecyl acids or their derivatives, or double unsaturated eicodienoic acids or their derivatives, and n ranges from 2-10; and

diethanolamides having the formula

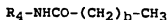


where R_2 is caprylic, lauric, myristic, stearic, or linoleic acid or their derivatives.

10 $R_3\text{-COO}(\text{C}_2\text{H}_4\text{O})_m\text{H}$

where R_3 is lauric, myristic, palmitic, stearic, or oleic acids or their derivatives, double unsaturated octadecyl acids or their derivatives, or double unsaturated eicodienoic acids or their derivatives and m ranges from 2-4;

long chain acyl hexosamides having the formula



where b ranges from 10-18 and R_4 is a sugar molecule selected from a group consisting of glucosamine, galactosamine, and N-methylglucamine;

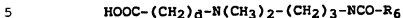
long chain acyl amino acid amides having the formula

- 33 -



where c ranges from 10-18 and R_5 is an amino acid side chain;

long chain acyl amides having the formula



where R_6 is an acyl chain having 12-20 carbons and not more than two unsaturations, and d ranges from 1-3;

polyoxyethylene (20) sorbitan mono- or trioleate;

10 polyoxyethylene glyceryl monostearate with 1-10 polyoxyethylene groups;

glycerol monostearate;

sarcosinamides having the formula



where R_7 is selected from the group consisting of single-chain carbonyl derivatives of C_{12} - C_{20} fatty acids; and

betaines having the formula



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where R_g is selected from the group consisting of long-chain fatty acid esters.

12. The method of claim 11 wherein said lipid phase further comprises a sterol.

5 13. The method of claim 12 wherein said sterol is selected from the group consisting of cholesterol, hydrocortisone, and derivatives and chemical analogues thereof.

14. The method of claim 11 wherein said lipid
10 phase further comprises a charge-producing agent.

15. The method of claim 14 wherein said charge-producing agent is selected from the group consisting of carboxylic acids, dicetyl phosphate, cetyl sulfate, phosphatidic acid, phosphatidyl
15 serine, quaternary ammonium salts, and mixtures and derivatives thereof.

16. The method of claim 11 wherein said first lipid is selected from the group consisting of phosphatidylcholines, phosphatidylethanolamines,
20 phosphatidylserines, inositolphosphatides, sphingomyelins, ceramides, cerebrosides, gangliosides, sulfatides, and derivatives and chemical analogues thereof.

17. The method of claim 11 wherein said hybrid
25 paucilamellar vesicles are oil-filled hybrid paucilamellar vesicles, said method comprising the further steps of:

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forming an oily phase by blending any oil dispersible material to be encapsulated in said vesicles in a water-immiscible oily solution; and

blending said lipid phase and said oily
5 phase to form a lipophilic phase both shear mixing with said aqueous phase to form said vesicles.

18. The method of claim 17 wherein said water-immiscible oily solution is selected from the group consisting of oils, waxes, natural and
10 synthetic triglycerides, acyl ethers, petroleum derivatives, and their derivatives and chemical analogues.

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US90/05294

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all *)		
According to International Patent Classification (IPC) or to both national classification and IPC		
INT. CL. (5): A61K 9/127, 9/122; B01D 13/02		
U.S. Cl. 264/4.1; 424/450; 428/402.2; 436/829		
II. FIELDS SEARCHED		
Minimum Documentation Searched *		
Classification System	Classification Symbols	
U.S. Cl.	264/4.1; 424/450; 428/402.2; 436/829	
Documentation Searched other than Minimum Documentation to the extent that such documents are included in the fields searched *		
III. DOCUMENTS CONSIDERED TO BE RELEVANT *		
Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages †	Relevant to Claim No. ‡
X	US, A, 4,217,344 (Vanlerberghe et al) 12 August 1980 See examples 1,2,13,19,23 & 25; and Col.4, lines 22,27; and Col.4, line 56 - Col. 5, line 44	1-6,10-16
X	US, A, 4,247,411 (Vanlerberghe et al) 27 January 1981 See examples 2,3,6&8; and Col.3, line 25 - Col.4, line 41.	1-6,10-16
X	US, A, 4,448,765 (ASH et al) 15 May 1984 See examples 1-6; and Col.4, line 62 - Col.5, line 40.	1-6,10-16
X	"Les Niosomes" (Handjani-Vila et al) 1985, pages 297-313. See especially pages 302-309.	1-6, 10-16
X	US,A, 4,911,928 (Wallach) 27 March 1990 See patent claims 1-12.	7-9,17,18
X	US, A, 4,942,038 (Wallach) 17 July 1990 See patent claims 1-4, 18 and 19.	7-9
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17 December 1990	29 JAN 1991	
International Searching Authority	Signature of Authorized Official: NGOC-HO	
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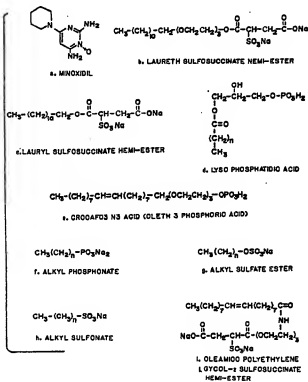


INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(54) Title: NON-CRYSTALLINE MINOXIDIL COMPOSITION AND METHOD OF APPLICATION**(57) Abstract**

An aqueous, non-crystalline minoxidil composition for topical use. The composition contains minoxidil complexed with an amphipathic compound having a pK less than about 5 and containing a single lipophilic chain moiety and a sulfate, sulfonate, phosphate and phosphonate polar moiety. The composition may be formulated in ointment form, in an aqueous vehicle, or dispersed in a fluorochlorocarbon solvent, for spray delivery from a self-propelled spray device. The minoxidil in the composition remains in non-crystalline form for a period of at least several hours after application to the skin, thus overcoming the prior art problem of the minoxidil tending to revert to an insoluble crystalline form when prior art compositions are applied to the skin.



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NON-CRYSTALLINE MINOXIDIL COMPOSITION AND METHOD OF APPLICATION

1. Field of the Invention

10 The present invention relates to a topical minoxidil composition, and in particular, to a non-crystalline composition which can be applied in spray or ointment form.

2. Background of the Invention

15 Minoxidil is an arterial dilator which has been used, in oral form, in the treatment of hypertension to lower blood pressure. More recently, the drug has been shown to stimulate new hair growth, when applied topically, in cases of male pattern
20 baldness. Initial clinical studies with a topical form of the drug indicate that reversal of male pattern baldness is most favorable in younger men, and where recent hair loss has occurred, but that new hair growth is observed in a significant percentage of older men
25 and/or where in cases of long-term baldness.

The drug itself is a piperidinyl pyrimidine compound which is poorly soluble in water and in most water-immiscible organic solvents such as chloroform. Heretofore, minoxidil has been formulated, for topical
30 use, in an ethanol-based ointment vehicle containing ethanol, propylene glycol and water. The solubility of the drug in pure propylene glycol is between about 7-9% by weight, and in an ethanol/propylene glycol/water vehicle, only about 2%. On drawback of the formulation

is the tendency of the minoxidil to revert to an insoluble crystalline form when applied to the skin, as the ethanol solvent evaporates. Whether due to the tendency of the drug to crystallize or other factors, the minoxidil formulation shows relatively inefficient uptake by the skin. Another limitation is the limited solubility of the drug in the ethanol/propylene glycol/water vehicle. Further, evaporation of ethanol, when the formulation is applied to the skin, leaves a viscous propylene glycol/water residue which may be objectionable to many users. The drug is poorly soluble in water and practically insoluble in lipophilic solvents, such as chloroform. Therefore, predominantly water-based or propellant-solvent formulations have not been feasible heretofore.

European patent application No. 177,223 discloses a liposomal minoxidil composition in which minoxidil is present (1) in solution form possibly in a supersaturated state, either encapsulated in lipid vesicles, or in the aqueous or lipid phases of a liposome suspension, and (2) in a finely divided crystalline (solid) form both within and outside the lipid vesicles. Preferred embodiments of the composition are formed by hydrating a minoxidil lipid film containing a saturated phosphatidylcholine (PC), such as dipalmitoylphosphatidylcholine (DPPC), cholesterol, and minoxidil with an aqueous solution containing minoxidil in ethanol/propylene glycol and water. Minoxidil is present at a final weight concentration of between about 1.2-3%. The composition was found to contain liposomes of various sizes between about 1 μ to 15 μ , and minoxidil crystals. Although the formulation is reported to increase drug uptake by the epidermis when applied topically to skin, it has the

same limitation as the above non-liposomal formulation in that the drug is applied to the skin largely in crystalline form.

5 3. Summary of the Invention

It is one general object of the present invention to provide a non-crystalline minoxidil composition that can be formulated in a water-based or lipophilic-solvent vehicle.

10 Another object of the invention is to provide such a formulation which gives enhanced transdermal penetration of the drug.

The invention includes a non-crystalline minoxidil composition in which minoxidil is complexed with an amphipathic compound having a pK of less than about 5, and containing a single lipophilic chain and a polar head moiety selected from a sulfate, sulfonate, phosphate, or phosphonate free acid. The molar ratio of the amphipath to minoxidil is at least about 1:1, and
20 the composition has a preferred pH between about 4-6. Preferred amphipathic compounds include sulfosuccinic acid hemiesters and alkyl phosphonates and phosphate esters, and exemplary sulfosuccinic acid hemiesters include ethoxylated sulfosuccinic acid hemiesters, such as the free acids of laureth, lauryl or oleamido-
25 polyethylene glycol sulfosuccinate.

In one embodiment, the composition is formulated to include vesicle-forming lipids, such as PC, to form a minoxidil/liposomal suspension. The
30 vesicle-forming lipids are preferably included at a molar ratio of between about 1:1 and 4:1 lipid to minoxidil.

In another embodiment, the composition is formulated to include surfactant components, to form an

emulsion or stable microemulsion/minoxidil composition. The minoxidil/amphipath complex may also be formulated in a micellar dispersion.

- The minoxidil composition may be administered
- 5 in either spray or ointment form. A convenient spray formulation includes the minoxidil/ester complex dispersed in a chlorofluorocarbon propellant solvent. Phospholipid, such as PC, may be added to achieve a molecular monodisperse form of the drug in the solvent.
- 10 The minoxidil spray is directed against the topical area of interest, to deposit (with solvent evaporation) a noncrystalline minoxidil composition on the skin.

- In a cream or ointment formulation, the minoxidil composition is dispersed in non-crystalline
- 15 form in an aqueous medium, in the presence of absence of suspended liposomal or lipid-emulsion particles. The formulation typically contains 1-6% minoxidil, at least an equal molar amount of the amphipathic compound, and between about 60-90% water. In a liposomal formulation,
- 20 the vesicle-forming lipids are present at a weight ratio of lipids to minoxidil of between about 1:1 and 4:1, where the transdermal uptake of minoxidil is reduced at higher lipid to drug ratios.

- More generally, this composition includes a
- 25 noncrystalline minoxidil composition in which minoxidil is (a) present at a weight concentration of between about 1-6%; (b) complexed with an amphipathic compound having a pK less than about 5, at an amphipath:minoxidil molar ratio of at least about 1:1; (c) dispersed in an
- 30 aqueous or lipophilic-solvent carrier, and (d) maintained in noncrystalline form for a period of at least several hours after application to the skin. The amphipathic compound is preferably one which promotes transdermal uptake of the drug.

Also forming part of the invention is a method for applying minoxidil topically in a substantially noncrystalline form which remains in non-crystalline form at least several hours after application to the skin. The method includes complexing the minoxidil with an amphipathic compound having a pK less than about 5, and containing a single lipophilic chain moiety and a polar head moiety selected from a sulfate, sulfonate, phosphate, or phosphonate free acid. Specific formulations, and methods of application of the complex to the skin are as described above.

These and other objects and features of the invention will become more fully apparent when the following detailed description of the invention is read in conjunction with the accompanying drawings.

Brief Description of the Drawings

Figure 1 shows the molecular structure of minoxidil (1a) and the structures of several amphipathic compounds which promote solubilization of minoxidil in aqueous and lipophilic solvents and lipid bodies, and enhance transdermal uptake of the drug (1b-1h);

Figure 2 shows the molecular structures of several amphipathic compounds which promote solubilization of minoxidil uptake, but do not enhance skin penetration of the drug;

Figure 3 is a plot showing changes in minoxidil solubility, in a 20% laureth sulfosuccinate mixture, as a function of pH;

Figure 4 is a plot showing the increase in minoxidil solubility with increased concentrations of laureth sulfosuccinate in a pH 5.0 mixture;

Figure 5 shows transdermal uptake curves, over a 24 hour period, for control (open squares), 5%

minoxidil/sulfosuccinate (open diamonds), 2% minoxidil/sulfosuccinate (crosses), and 2% minoxidil/taurocholate (open triangles) compositions;

Figure 6 shows transdermal uptake curves for control (open squares) and a 1% minoxidil/phosphate monoester composition (crosses);

Figure 7 shows transdermal uptake curves for lauryl sulfosuccinate/PC liposome compositions containing either 2% (crosses) or 5% (open diamonds) minoxidil, a 1% minoxidil/cholesterol sulfate composition (open triangles), and the control composition (open squares);

Figure 8 shows transdermal uptake curves for a 2% minoxidil/oleamido-PEG-2/PC liposome formulation (open diamonds), 2% lauryl sulfosuccinate/PC liposomes (crosses), and the control formulation (open squares); and

Figure 9 shows transdermal uptake curves for a 2% minoxidil/lysoPA/PA composition (crosses), a 2% minoxidil/PA composition (open diamonds), and the control formulation (open squares).

Detailed Description of the Invention

25 I. Minoxidil Composition in Aqueous Medium

The minoxidil composition of the invention is formed by complexing minoxidil with the free acid of an amphipathic compound. The amphipathic compound acts to solubilize minoxidil in both aqueous and lipophilic solvents, and preferred compounds also enhance transdermal uptake of the drug. This section describes amphipathic compounds for use in the invention, and methods of preparing a water-soluble minoxidil

composition which is suitable for ointment and cream formulations.

A. Amphipathic Compounds

5 Figure 1 shows the molecular structure of minoxidil (1a). The piperidinyl pyrimidinediamine compound is relatively soluble in alcohol, but poorly soluble in water (about 0.2-0.3%), and practically insoluble in chloroform.

10 The figure also shows the molecular structure of several specific and general types of amphipathic compounds which promote solubilization of the drug in both aqueous and lipophilic solvents and, according to an important feature of the invention, also enhance
15 transdermal uptake of the drug. The amphipathic compounds in this class are characterized by (a) a single lipophilic chain moiety and a polar head group moiety selected from a sulfate, sulfonate, phosphate, or phosphonate free acid, where the pK of the compound is
20 no greater than about 5.0. The exemplary compounds shown in the figure include laureth sulfosuccinate hemiester (1b) and lauryl sulfosuccinate (1c), representative of sulfosuccinate hemiesters, lysophosphatidic acid (1d) and monoalkyl phosphate
25 esters, such as Crodafos™ N3 (1e), monoalkyl phosphonates (1f), monoalkyl sulfate esters, (1g), monoalkyl sulfonates (1h), and oleamido-PEG-2-sulfosuccinate (1i), representative of amido-linked
amphipaths.

30 As can be appreciated from the compounds shown in Figure 1, the lipophilic chain moiety may be a pure hydrocarbon chain, or may contain ether or other chain linkages, such as internal ester or amide linkages. The chains lipophilic chains preferably include at least

about 6-8 carbon atoms, and are typically at least about 12 atoms in length. The chains may be attached to the mineral acid head group through ester, ether, thioether, amide, or other stable linkages, as illustrated
5 variously in the Figure 1 compounds.

A second class of amphipathic compounds which have been examined herein are capable of solubilizing minoxidil in an aqueous medium, but fail to promote transdermal uptake of minoxidil. Several of the
10 compounds in this class are acidic vesicle-forming lipids, typically having charged phosphate or sulfate free acid head groups, a pK less than about 5, and diacyl, dialkyl or sterol lipophilic moieties. Exemplary compounds include the free acid forms of negatively
15 charged phospholipids, such as phosphatidylglycerol (PG), phosphatidylinositol (PI), and phosphatidic acid (PA), dialkyl phosphate compounds, such as dicetyl phosphate, and sterol mineral acids, such as cholesterol sulfate and taurocholic acid. Other phosphate, phosphonate,
20 sulfate, and sulfonate lipids containing two or more lipophilic chains or a sterol group through ester, ether, or amide linkages are also in this general class. Representative members of this class are illustrated in Figure 2. These are: taurocholic acid (2a), cholesterol
25 sulfate (2b), phosphatidic acid (2c), and phosphatidylglycerol (2d).

B. Preparing the Minoxidil Composition

According to an important property of the
30 amphipathic compounds, optimal solubilization of minoxidil by the amphipathic compound occurs at a pH of about 5 or less, where a significant fraction of the compound exists in free acid form. The solubility dependence of minoxidil on pH is illustrated in Figure

3, for a 20% by weight solution of laureth sulfo-
succinate (Figure 2a compound). Between pH 7.0 and
about 5.0, minoxidil solubility increases from about
1.5% to nearly 5%. Little improvement is seen as the pH
5 is lowered beyond about 4.5. For most purposes, a pH of
about 5 is preferred, since good solubility is achieved,
and skin irritation which may result from below-
physiological pH is minimized.

In a preferred method for preparing the
10 composition, a portion of the amphipath is converted to
a free acid form, and then "titrated" to the desired pH,
e.g., pH 5.0, with the metal salt form of the compound.
This approach is illustrated in Example 1, which
describes the preparation of a 20 weight % laureth
15 sulfosuccinate solution having a final pH of 5. Here
the disodium salt of the compound is converted to the
free acid form by passage through a cation exchange
resin. Mixing the free acid with the disodium salt, at
a ratio of about 1:3, yields a pH 5.0 solution suitable
20 for solubilization of the minoxidil. It is appreciated
that the free acid and salt components effectively
buffer the solution at the selected pH, obviating the
need for additional buffering components.

Alternatively, the compound may be converted
25 to or supplied in free acid form, then adjusted to the
selected pH with a suitable base, such as NaOH. In
another approach, the dispersion containing the
amphipathic compound can be acidified, typically to
between pH 1-2 before complexing with minoxidil. After
30 forming the desired complex, the dispersion can be
titrated back to a suitable pH, typically between 4-6.

Optimal solubilization of the minoxidil in an
aqueous formulation requires a molar concentration of
amphipathic compound to minoxidil of at least about 1:1,

10.

and molar ratios of between 1:1 and 1:5 are typical. Figure 4 illustrates the increasing solubility of minoxidil with increasing concentration of amphipath in an aqueous solution at pH 5.0. Details are given in
5 Example 4. As seen from the figure, minoxidil solubility up to about 5 percent by weight was achieved at the highest amphipath concentration.

The minoxidil composition is preferably formed by adding dry minoxidil to the aqueous solution of
10 amphipath, prepared as above, to a desired pH and amphipath concentration. Typically, the solution is warmed to about 50°C, and the minoxidil is added slowly with stirring. When the minoxidil is completely dissolved, the solution is cooled and the pH adjusted,
15 if needed. The general method is illustrated in Examples 1 and 2, for the preparation of laureth sulfosuccinate/minoxidil compositions; in Example 5, for the preparation of a Crodafos™/minoxidil composition; and in Example 6, for the preparation of a taurocholic
20 acid/minoxidil composition. All of the compositions gave clear aqueous solutions.

As indicated above, the composition of the invention includes minoxidil in a substantially non-crystalline or molecular monodisperse or dissolved form.
25 These terms are defined herein to indicate that the minoxidil composition is substantially free of crystalline minoxidil, as judged, for example, by examination by polarization microscopy. It will be appreciated that the minoxidil may be present in a
30 microdispersion, such as in micellar or microemulsion form, and/or as a soluble molecular binary drug/amphipath complex. Thus, in preparing the composition, complete drug solubilization is judged by

the absence of the drug in crystalline or micro-crystalline form.

The composition may include additional soluble or suspension components, such as metal chelators, preservatives, and/or conventional lipid, emulsifying or gelling agents used in formulating ointment and cream topical formulations. Exemplary metal chelators include EDTA and DTPA, and exemplary preservatives include propyl- and methylparaben. Agents suitable for formulating the composition in cream or ointment form are known.

The use of the aqueous composition for topical administration of minoxidil, and transdermal uptake characteristics of compositions containing each class of amphipathic compound, are considered in Section IV below.

II. Minoxidil Composition in Lipophilic Solvents

According to another important aspect of the invention, the above-described amphipathic compounds which promote solubilization of the drug in aqueous medium, also promote solubilization in lipophilic solvents, such as chloroform, in which the drug is otherwise practically insoluble.

The amphipathic compound used in preparing the minoxidil composition in a lipophilic solvent may be supplied in free form, or converted to a free acid by treatment with a cation-exchange resin, as above. Typically, however, when the compound is supplied is the salt form, the compound is most conveniently converted to a free acid by solvent extraction into the organic solvent phase of an acidified, two-phase extraction mixture, such as in the Bleigh-Dyer extraction procedure detailed in Example 7. In this example, the amphipathic

compound is shaken in an acidified chloroform/methanol/water mixture and extracted in free acid form from the lower chloroform phase.

The solution of amphipathic compound
5 containing the free acid form of the compound in a lipophilic solvent (such as the lower-phase extract from a BleighDyer extraction) is mixed with dry minoxidil to form a non-crystalline dispersion of minoxidil in the solvent. For many amphipathic compounds, such as those
10 described in Examples 7-11, dispersion of the drug in a nonflocculated form also requires addition of a vesicle-forming lipid, such as PC, as illustrated in Examples 7-9, or an emulsion-forming agent, such as illustrated in Example 10 and 11. Vesicle or emulsion-forming lipids
15 are also added to the solution when the composition is to be used in forming a liposomal or emulsion form of molecularly dispersed minoxidil, as detailed in Section III below. Solubilization of minoxidil in the lipophilic solvent containing an amphipathic compound
20 may also be achieved by addition of a co-solvent, such as an alcohol or glycol, to the mixture of minoxidil and amphipath in lipophilic solvent.

One application of the minoxidil/lipophilic solvent mixtures is for delivery of the drug in spray
25 form from a self-propelled atomizer system. Here minoxidil, the amphipathic compound and, if needed, a vesicle- or emulsion-forming agent are co-dissolved in a Freon chlorofluorocarbon solvent. Several fluorochloro-carbon propellant solvents have been used or proposed
30 for self-contained spray devices. Representative solvents includes "Freon 11" (CCl_3F), "Freon 12" (CCl_2F_2), "Freon 22" (CHClF_2), "Freon 113" ($\text{CCl}_2\text{FCClF}_2$), "Freon 114" ($\text{CClF}_2\text{CClF}_2$), and "Freon 115" (CClF_2CF_3), as

well as other fluorochloro substituted methyl and ethyl compounds.

5 The propellant solution is loaded in a conventional pressurized propellant spray device for delivering a metered amount of spray-dried minoxidil dispersed in the propellant. Since the spray system may require long-term storage of the solution components in the propellant solvent, the lipid components in the system should be selected for stability on storage, for
10 example by employing partially or totally saturated amphipathic and vesicle-forming lipid components.

In use, the propellant spray device produces a fine-particle spray of solubilized minoxidil which is directed against the skin area being treated. The spray
15 particles initially contain minoxidil complexed with the amphipathic compound and, if present, added co-solubilizing agent, dispersed in the propellant solvent. Rapid evaporation of the solvent, as the spray particles are ejected through the air, yields noncrystalline
20 minoxidil particles which form a layer of drug particles which substantially cover the sprayed skin area.

III. Minoxidil Composition in Lipid Bodies

25 According to another aspect of the invention, the minoxidil composition can be entrapped in non-crystalline form in both lipid emulsion particles and liposomes, providing additional advantages for topical administration of the drug. One of these advantages, in the case of liposomes, is the ability to modulate the
30 rate of drug release from the composition, by selection of the suitable vesicle-forming lipids. Another is the greater drug loading capacity of lipid particles. Liposomes and emulsion particles are both compatible with topical ointment and cream formulations, and in

fact are commonly added to skin creams as moisturizing agents. Liposomes may also be adapted for use with self-propelled spray systems, providing a convenient method of delivery of a non-crystalline, high-
5 concentration minoxidil composition.

Considering first the preparation of non-crystalline minoxidil liposomes, the vesicle forming lipids are preferably neutral phospholipids, such as PC, and may also include negatively charged phospholipids,
10 such as PG, phosphatidylinositol (PI), and phosphatidylserine (PS) which can function as the negatively charged amphipaths in the composition. For the reasons discussed above, however, the amphipathic compound used in the composition is preferably a single chain mineral
15 acid compound of the type which by itself would not form lipid bilayer vesicles upon hydration. Other liposomal lipids, such as cholesterol, may also be included.

Studies conducted in support of the present
20 invention indicate that minoxidil transdermal uptake can be modulated by the factors which affect the fluidity of liposome membranes, such as the extent of phospholipid acyl chain saturation. As a rule, transdermal uptake is is decreased by entrapment of the non-crystalline
25 composition in liposomes. Another factor which is important in rate of drug uptake is the ratio of vesicleforming lipids to minoxidil. Preferred weight ratios of phospholipid to minoxidil are between about 1:1, which gives relatively high transdermal uptake and
30 4:1, which gives quite low uptake.

The non-crystalline liposome composition can be formed by a variety of methods which are modifications of existing liposome preparation methods. For example, to prepare the liposomes by lipid

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hydration, a lipid solution containing minoxidil, the amphipathic compound, and PC, is prepared as above, by dissolving the lipid and minoxidil in the lower-phase solution of amphipath in free acid form. The resulting
5 solution of minoxidil, amphipath, and lipid are dried to a thin film, then hydrated with a suitable aqueous buffer. This hydration method is illustrated in Examples 7-9 below. Alternatively, a film of vesicle-forming lipids alone can be hydrated by a solution of
10 the non-crystalline minoxidil composition, formed as in Section I.

One preferred method of forming the liposome composition uses a novel lipid injection method described in co-owned patent applications for "High-
15 Encapsulation Liposome Processing Method", Serial No. 908,765, filed September 18, 1986, and "High-Concentration Liposome Processing Method", Serial No. 909,122, filed September 18, 1986. In this method, a solution of minoxidil, amphipathic compound, and
20 vesicleforming lipids in a preferably chlorofluorocarbon solvent is prepared as described above in Section II. This solution is injected into an aqueous medium under selected temperature and pressure conditions which lead to liposome formation. According to an important
25 feature of the method, solvent injection may be continued, with or without concomitant liposome sizing, until a liposome composition having the consistency of a thick paste is formed. The paste composition has the capability of high minoxidil loading, and also is
30 suitable as a cream or ointment without further processing.

The liposome composition may also be delivered in dried particle form from a self-propelled spray device. Here the liposomes, formed according to above

5 methods, are dried, by spray drying, then suspended in a chlorofluorocarbon propellant solvent. Methods for spray drying liposomes and forming stable liposome-particle suspensions in several Freon propellants, have been described in co-owned U.S. patent application for "Liposomes for Inhalation", filed February 2, 1987, and similar methods are applicable to the liposomal composition of the present invention. Studies conducted in support of the just-cited patent applications have
10 examined the stability and size characteristics of spray dried liposomes in several Freon propellants. Good liposome stability, as measured by microscopic examination of the liposomes and retention of encapsulated material was seen with Freons 12, 113, 114,
15 and 115.

The suspension of dried minoxidil liposomes in propellant solvent can be administered in metered dose spray form from a conventional pressurized spray device such as used above for delivery of a Freon dispersion
20 solution of minoxidil/amphipath components.

Methods for producing a non-crystalline lipid emulsion composition, according to the invention, may similarly follow standard preparative methods, with modification to include the amphipathic compound needed
25 for minoxidil solubilization. Examples 10A and 10B illustrate methods for forming emulsions of lysoPA/PA and PA alone in a Tween-20™ emulsion, respectively. Briefly, a Bleigh-Dyer solvent extraction containing the free acid form of the lysoPA/PA mixture or PA alone was
30 added to minoxidil, and the lipid solution dried to a thin film. Hydration of the lipid film with an aqueous buffer containing 30% Tween-20™ yielded an aqueous non-crystalline emulsion.

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The minoxidil composition of the present invention may also be formulated as a stable microemulsion, i.e., a clear, thermodynamically stable emulsion. Typically, the microemulsion is formed by first producing a water-in-oil microemulsion composed of conventional surfactant and oil-phase components and the amphipathic compound needed for minoxidil solubilization. This is done conventionally, e.g., by combining the oil-phase, surfactant and amphipath components at a suitable temperature, e.g., 60°C, with stirring. After the mixture clears, it is acidified (if it is not otherwise in acid form), and minoxidil is added slowly with stirring until the mixture becomes clear again. The final clear microemulsion can be adjusted to pH 5.0 without destabilizing the dispersion. Example 11A illustrates a 4% water-in-oil microemulsion formed according to this general procedure.

An oil-in-water microemulsion can be formed readily by adding water dropwise to the above microemulsion, until a desired minoxidil concentration is reached. This procedure is illustrated in Example 11B below, which describes preparation of a 2% minoxidil oil-in-water microemulsion. An oil-in-water emulsion may also be prepared directly by increasing the concentration of water included in the microemulsion formed before addition of minoxidil.

Alternatively, the emulsion may be formulated in a concentrated, viscous or paste-like form for topical administration, giving the same advantages of high loading available with the liposomal formulation. Alternatively, the emulsion composition can be produced in a self-propelled device by dissolving the minoxidil/amphipath/emulsion lipid components in a Freon solvent, as above, and delivering the components in

spray form. Non-crystalline minoxidil/lipid particles are formed during rapid evaporation of the propellant solvent.

5 IV. Utility

A. Transdermal Uptake Characteristics

10 The transdermal uptake characteristics of several exemplary minoxidil compositions prepared according to the invention have been examined, as outlined generally in Example 12. Briefly, a small isolated skin patch is sealed between upper and lower chambers of a transdermal cell, and an aliquot of the selected composition (spiked with radiolabeled

15 minoxidil) is applied to the upper surface of the patch. The lower chamber holds a reservoir buffer which is in contact with the lower surface of the skin, and which is circulated through the lower chamber by a constant-rate pump. As drug penetrates the skin patch, it is captured

20 in the lower reservoir, and pumped out of the chamber into assay vials for scintillation counting. Transdermal uptake (drug penetration) is typically measured for over a 24 hour period.

The preparation of the several compositions

25 studies is detailed in Examples 1, 2, and 5-11. The control drug composition used in the studies is a 2% minoxidil composition in an ethanol/propylene glycol/water vehicle. The transdermal uptake of this control formulation, over a 24 hour period, is shown by

30 the open squares in Figure 5. The cumulative amount of drug taken across the skin in the 24 hour period is less than about 1% of the total applied to the skin.

Also shown in the figure are the transdermal uptake curves for a soluble 2% minoxidil/laureth

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5 sulfosuccinate composition (crosses), and a soluble 5% minoxidil laureth sulfosuccinate composition (open diamonds). As seen, both of the soluble laureth sulfosuccinate compositions gave higher transdermal penetration rates than the control composition, expressed as μg drug penetrating/ cm^2 of skin. The total cumulative drug uptake for the 2% composition is about 6% of the total applied to the skin.

10 Interestingly, the 2% laureth sulfosuccinate composition, which contains about 8% by weight of the laureth sulfosuccinate, gave a significantly higher transdermal uptake than the 5% composition, which contains about 25% by weight of the amphipath. This result may be due to the greater viscosity of the 5%
15 composition, and/or interactions between the surfactant and skin which are less inhibitory in the 2% composition.

Figure 5 also shows the transdermal uptake for a soluble 2% minoxidil/taurocholic acid composition
20 (open triangles). It is evident that taurocholic acid does not promote the uptake of minoxidil across the skin, even though it is effective in solubilizing the drug in an aqueous formulation.

Figure 6 shows similar transdermal uptake data
25 comparing a minoxidil/crofadol composition prepared according to the invention with the above control composition. The transdermal uptake for the composition of about $700 \mu\text{g}/\text{cm}^2$ is nearly three times that of the 2% laureth sulfosuccinate composition and about 35 times
30 that of the control material. The total amount of drug material passing through the skin in a 24 hour period is about 35% of the total added.

The transdermal uptake characteristics of several liposomal and emulsion compositions were also

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examined, and compared with the above minoxidil control composition. Figure 7 shows transdermal uptake curves for 2% (crosses) and 5% (open diamonds) minoxidil/lauryl sulfosuccinate/PC liposome suspension formed as in
5 Example 7. The data show enhanced transdermal uptake when compared with the control formulation (open squares). It is noted that, in contrast to the results observed for the soluble laureth sulfate composition, the higher percent composition (5% minoxidil) gave
10 greater transdermal uptake. Both liposome formulations gave about 5% total drug penetration over the 24 hour test period. It is also noted that the best liposomal formulation gave higher drug penetration (about 350 μg drug/ cm^2) than the best laureth sulfosuccinate
15 composition (about 240 μg drug/ cm^2).

The figure also shows transdermal uptake for a 1% minoxidil/cholesterol sulfate/PC liposome composition. This composition thus differs from the ones just discussed in that cholesterol sulfate has been
20 substituted for lauryl sulfosuccinate. As seen, virtually no transdermal uptake of minoxidil occurred during the 24 hour test period.

In Figure 8, the transdermal uptake of the above 2% minoxidil/lauryl sulfosuccinate/PC liposomes
25 (crosses) is compared with that of 2% minoxidil/oleamido PEG-2 sulfosuccinate/PC liposomes (open diamonds). As observed, the latter composition gave substantially higher transdermal uptake than either 2% or 5% minoxidil/lauryl sulfosuccinate/PC liposome
30 compositions.

Finally in Figure 9 are shown transdermal uptake curves for suspensions formed from 2% minoxidil and either lysoPA/PA (crosses) or PA alone (open diamonds), according to the preparative methods of

Examples 10 and 11, respectively. The control composition is indicated by open squares. PA alone gives very poor drug uptake, whereas with the addition of lysoPA, uptake is enhanced slightly above the control level.

Considering the data as a whole, it is seen that enhanced transdermal uptake is present in each composition where the amphipathic compound used to solubilize minoxidil contains a single lipophilic chain and a sulfosuccinate (sulfate), phosphonate, or phosphate polar head. In all cases where the lipophilic moiety of the amphipath was either a sterol or included more than a single lipophilic chain, transdermal uptake was severely limited.

B. Drug Administration

The composition of the invention may be administered topically in a water-base cream, ointment, or gel form. Several factors contribute to cosmetic advantages of the water-base composition. High drug loading up to 5% or more in non-crystalline form is possible, and the drug remains in a non-crystalline form up to several hours or more after administration, since solvent evaporation is relatively slow and because evaporation can occur without leading to drug crystallization. Since the composition contains little or no alcohol, it can be applied without stinging.

The drug is efficiently taken up by the skin, by virtue of the selected amphipath, and different rates of uptake can be achieved by varying the drug or amphipath concentration, and by selection of different amphipathic compounds.

The water-base composition may additionally contain liposome or lipid-emulsion particles in which

the drug can be entrapped in non-crystalline form. In one embodiment, a concentrated liposomal composition having desired a desired cream or ointment consistency can be formulated using a novel solvent injection system. The liposome formulation can have high loading, and rate of drug uptake can be modulated by choice of lipid components and relative molar amounts of lipid and drug. The lipid formulation is also expected to have the known moisturizing benefits of topical lipid formulations.

In another embodiment, the composition is dispersed in a chlorofluorocarbon solvent for delivery in spray form. The spray form has many advantages of the water-base formulation, including high drug loading and enhanced drug uptake. Additionally, the spray composition has the advantage that it can be applied in a more convenient manner and without matting the hair in the treated scalp region.

The following example illustrate methods of preparing non-crystalline minoxidil compositions according to various embodiments of the invention, and compares transdermal penetration characteristics of the various compositions. The example are intended to illustrate, but not limit the scope of, the invention.

Materials

Disodium laureth sulfosuccinate was obtained from Sherex (Dublin, CA), and supplied under the trade name "Varsulf SBFA-30" or from Mona Industries (Patterson, NJ), and supplied under the trade name "LEO-40"; disodium lauryl sulfosuccinate, from Mona Industries; disodium salt of taurocholic acid, from Sigma Chemical Co. (St. Louis, MO); oleamido-

polyethylene glycol-2 sulfosuccinate, disodium salt, from Mona Industries; Crodafos™ N3 acid (oleth 3 phosphate), from Croda, Inc. (Fullerton, CA); phosphatidic acid (PA), from Avanti Polar Lipids, Inc. (Birmingham, AL); Tween-0 from J.T. Baker (Phillipsburg, NJ); and cholesterol sulfate, from Sigma Chemical Co. (St. Louis, MO). Minoxidil, USP, was obtained from Upjohn (Kalamazoo, MI); methylparaben and propylparaben, from Sigma Chemical Co (St. Louis, MO); diethylenetri aminopentaacetic acid (DTPA), from Aldrich (Milwaukee, WI); and partially hydrogenated egg phosphatidylcholine (PC), from Asahi (Tokyo, Japan). MES (morpholinoethane sulfonic acid) was obtained from Sigma (St. Louis, MO). AG 50W-X8 cation exchange resin were supplied by Bio-Rad (Rockville Center, NY). Polyethoxylated (C8-C10) glycerides (Labrosol™), stearyl alcohol ester of stearic acid (isostearate D'isostearique), and polyglycerol isostearate (plurol isostearique) were obtained from Gattefosse™ (Elmsford, NY).

Example 1

5% Minoxidil/Laureth Sulfosuccinate Composition

A. Preparing the free acid (laureth sulfosuccinic acid) from disodium laureth sulfosuccinate.

250 ml distilled water was added to 750 ml of a 40% (wt/vol) solution of disodium laureth sulfosuccinate to make one liter of 30% solution. This solution was passed over a prepared column packed with approximately 270 g of AG50W-X8 cation exchange resin that has been converted to the hydrogen ion form. The eluate was collected as a single fraction after the pH

of the eluate dropped to 1.3. This eluate was the free acid, lauryl sulfosuccinic acid.

B. Complexing minoxidil with the hemiester sulfosuccinate.

5 800 ml of the AG50W-X8 eluate from above was mixed with 2,400 ml of a 30% (wt/vol) solution of disodium laureth sulfosuccinate. The resulting solution was heated to about 50°C and 192 grams minoxidil was
10 added slowly with stirring. After complete dissolution of the minoxidil occurred, the solution was cooled to room temperature, and the pH adjusted to 5.0 ± 0.1 . Distilled water sufficient to bring the volume to 3,840 ml was added, yielding a clear, 5% minoxidil/25%
15 surfactant solution which is free of microcrystals, as judged in the polarizing microscope (630x magnification).

The pH of the final dispersion can be varied by changing the ratio of laureth sulfosuccinic acid to
20 disodium laureth sulfosuccinate. A 1:3 ratio (as above) after minoxidil dissolution gave a pH of about 4.7 ± 0.2 .

Example 2

25

2% Minoxidil/Laureth Sulfosuccinate Composition

A 2% dispersion of solubilized minoxidil and the free acid of laureth sulfosuccinate was prepared substantially as in Example 1, with the following
30 modifications: The AG50W-X8 column was prepared with 100 g. Two hundred fifty ml of 40% wt/vol disodium laureth sulfosuccinate was diluted to 20% wt/vol surfactant by adding 250 ml distilled water. This solution was passed over the AG50W-X8 cation exchange

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column and the free acid eluate collector. Four hundred ml of free acid solution was combined with 1,200 ml of 20% disodium laureth sulfosuccinate and 2.0 liters distilled water. The mixture was heated to 50°C and 80 grams minoxidil was added slowly with mixing. After minoxidil dissolution, other excipients may be added. The mixture was cooled to room temperature, and the pH adjusted to about 5.3 ± 0.1 . Distilled water was added to give 4.0 l of a clear dispersion containing 2% solubilized drug and 8% laureth sulfosuccinate.

Example 3

Minoxidil Solubility: pH Dependence

A 20% solution of the free acid of laureth sulfosuccinate in distilled water was prepared as described in Example 1A. More acidic solutions of laureth sulfosuccinate were prepared by increasing the proportion of free acid in the free acid/disodium salt mixture, and more basic forms, by decreasing the ratio. The different-pH solutions were each heated to about 50°C and dry minoxidil containing tritiated minoxidil was added slowly with stirring until minoxidil saturation was achieved. The dispersions were cooled overnight at 4°C and centrifuged. The concentration of minoxidil in the clear solution was determined by scintillation counting. The results, expressed in mg minoxidil/ml laureth sulfosuccinate solution, are plotted in Figure 3 for two separate experiments. As seen, minoxidil solubility is very low at pH 7.0, and increases linearly to a maximum at a pH about 4.5-5.0.

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Example 4Minoxidil Solubility:Dependence on Amphipath Concentration

5 Solutions of the free acid of laureth
sulfosuccinate, at concentrations of 0, 5%, 10%, 15%,
20%, and 25% by weight in distilled water were prepared
as in Example 1A. Each solution was heated to about
10 50°C, and radiolabeled minoxidil was added slowly with
stirring until minoxidil saturation was achieved, this
being monitored as described in Example 3. The pH of
each solution was adjusted to about pH 5 prior to
centrifugation and scintillation counting. The results,
expressed in mg minoxidil/ml laureth sulfosuccinate
15 solution, are plotted in Figure 4. Minoxidil solubility
in the absence of the amphipath is about 3 mg/ml, or
0.3%. With increasing concentrations of the laureth
sulfosuccinate, up to 25 weight percent, the solubility
if minoxidil increases up to about 50 mg/ml, or 5% at
20 pH 5.

Example 5Minoxidil/Crodafos™ Composition

25 Five milliliters of Crodafos™ N3 acid (the
oleth-3 phosphate) was obtained in free acid form and
diluted in 5 ml distilled water: 10 ml punctilious
ethanol. The solution was heated to 37°C, 2 g minoxidil
was added slowly with stirring until dissolution. The
30 mixture was diluted with 180 ml of aqueous solution
buffered by MES at pH 5.5. Examination of the
composition with a polarizing microscope showed no drug
crystals in the dispersion containing 1% minoxidil and
5% Crodafos™ N3 acid.

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Example 6Minoxidil/Taurocholic Acid Composition

5 Taurocholic acid, sodium salt, was converted
to a free acid form by the procedure of Example 11.
About 100 mg by weight of the free acid form was mixed
with 20 mg of dry minoxidil in 1 ml pH 5.5 buffer,
yielding a clear solution with a final minoxidil
10 concentration of about 2% by weight. Stirring was
continued until a clear solution was obtained, after
which the pH of the solution was readjusted to 5.0. No
crystals were observed on examination of the composition
with a polarizing microscope.

15

Example 7Minoxidil/Lauryl Sulfosuccinate/Liposome Composition

20 This example describes the preparation of a
minoxidil/lauryl sulfosuccinate/liposome composition.
The free acid form of disodium lauryl sulfosuccinate was
formed by the Bleigh-Dyer extraction procedure, as
follows: A first solvent mixture was prepared by mixing
8 ml 1 N HCl, 20 ml methanol, and 10 ml chloroform in a
25 250 ml separatory funnel. To this solvent was added 400
mgs of the disodium lauryl sulfosuccinate, which was
dissolved by vigorous shaking. A second solvent mixture
containing 7 ml HCl, 10 ml chloroform, and 3 ml
distilled water was added to the funnel, which was then
30 shaken vigorously, and allowed to phase separate.

The lower chloroform phase (containing the
bulk of the free acid form of the lauryl sulfosuccinate)
was collected in a 250 ml round bottom flask containing
580 mgs partially hydrogenated PC (PHPC) and 200 mgs of

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minoxidil, both in dry form. The flask was swirled until both of the dry components were in solution, and to this solution was added butylated hydroxy toluene (BHT) in chloroform to a final concentration of about 1 mM.

The upper phase in the separatory funnel was reextracted with 5 ml chloroform and 1 ml methanol by vigorous shaking, and the lower phase which formed on standing was collected in the flask containing the minoxidil/lipid solution. The solvent in the flask was removed by rotary evaporation, yielding a thin lipid film. The dried material was further lyophilized for 1/2 hr to insure complete solvent removal.

The final minoxidil/liposome suspension was formed by hydrating the lipid film with 10 ml of MES buffer, pH 5.5, containing 0.01 % DTPA, using a mechanical "wrist" shaker. Shaking for 1 hour with the flask maintained at 50° C over a water bath was sufficient to produce complete hydration, as judged by the uniform appearance of the liposome suspension. The pH of the suspension, which had dropped to about 4 during the hydration step, was raised to 5.0 with 5 N NaOH. Microscopic examination of the liposome suspension showed a heterogeneous-size population of spherical vesicles. No polarizing crystals were noted.

Example 8

Minoxidil/Oleamido Sulfosuccinate/Liposome Composition

Oleamido-(2 polyethylene glycol)-sulfo-succinate was converted to the free acid form by Bleigh-Dyer extraction procedure, substantially as described in Example 7. The first lower chloroform phase was collected in a 250 ml round bottom flask

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containing 580 mgs partially hydrated PC (PHPC) and 200 mgs of minoxidil, both in dry form. To this was added the second lower extraction phase, as above, and the amphipath/minoxidil/lipid solution was taken to dryness with rotary evaporation and lyophilization.

The final minoxidil/liposome suspension was formed by hydrating the lipid film with 10 ml of MES buffer, pH 5.5, containing 0.01 % DTPA, under hydration conditions used in Example 7. The pH was adjusted to 5.0 after hydration was completed. Microscopic examination of the liposome suspension showed a heterogeneous-size population of spherical vesicles. No polarizing crystals were noted.

Example 9

Minoxidil/Cholesterol Sulfate/Liposome Composition

Cholesterol sulfate was converted to the free acid form by cation exchange chromatography in a methanol/chloroform/water (5:4:1) solvent. The free acid form (120 mgs) was dissolved in 2 ml of chloroform, and this solutions was added to a round bottom flask containing 40 mg minoxidil and 370 mgs of PC. The flask was gently agitated until the components were completely dissolved. The solution was dried to a thin lipid film in a round bottom flask with rotary evaporation and lyophilization, as above. A liposome suspension was prepared as in Example 8. No minoxidil crystal were observed in the suspension.

Example 10A. Minoxidil/LipoPA-PA Composition

5 A mixture of lipophosphatidic acid (lipoPA) and PA was formed by long term storage of pure PA at 4°C. The mixture was confirmed with thin layer chromatography as containing significant portions of both PA and lipoPA (Figure 2i). One hundred ten mg of the mixture was converted to the free acid form by the
10 Bleigh-Dyer extraction procedure above, and the combined lower-phase extracts were added to a round bottom flask containing 30 mg minoxidil. The flask was gently agitated until the drug was completely dissolved. The solution was dried to a thin lipid film in a round
15 bottom flask with rotary evaporation and lyophilization, as above.

The final minoxidil/lysoPA/PA liposome suspension was formed by hydrating the lipid film with 10 ml of MES buffer, pH 5.5, also containing 30%
20 Tween-0, under hydration conditions used in Example 7. The pH was adjusted to 5.0 after hydration was completed. Microscope examination of the suspension showed a very small (< 1 micron diameter) particles. No polarizing crystals were noted.

25

B. Minoxidil/PA Composition

Freshly obtained PA was examined by thin layer chromatography for purity, and only minor contaminants were observed. One hundred ten milligrams PA was
30 converted to the free acid form by the Bleigh-Dyer extraction procedure above, and the combined lower-phase extracts were added to a round bottom flask containing 30 mg minoxidil. An suspension was formed by hydration of the dried-film lipids, as in Example 9. No minoxidil

crystals were observed when examined with polarization microscopy.

Example 11

Minoxidil Microemulsions

5

A. Water-in-oil Microemulsion

- Labrasol™ (6.6 g), isostearate D;isostearique (0.8 g), plurol isostearique (2.8 g) (all obtained from Gattefosse'), and disodium laureth sulfosuccinate (4.0 g) and 2.03 g distilled water were mixed together in a 50 ml glass beaker, and the mixture stirred in a 54°C water bath until clear. The pH of the mixture was lowered to between 1-2 with a 1 N HCl. Minoxidil (0.8 g) was slowly added to the mixture at a temperature of about 56°C. Addition was complete after 10 minutes. After an additional 10 minutes of incubation, at a final temperature of about 60°C, the suspension was clear. The pH was then raised to about 5.0 with 5 N NaOH.
- 20 Incubation with stirring was continued for another 15 minutes, to a final temperature of about 64°C. The material was then allowed to cool to room temperature, and a final adjustment to pH 5 was made. The final dispersion was a clear, yellow oily microemulsion
- 25 containing 4% (by weight) minoxidil. The maximum amount of minoxidil which can be added, consistent with a clear microemulsion, is between about 5-7% by weight.

B. Oil-in-water Microemulsion

- 30 Distilled water was added dropwise to 10.57 g of the 4% minoxidil water-in-oil emulsion from part A above. A total of about 12 g of DW was added, to a final minoxidil concentration of about 2%. As DE was added, the clear oily suspension became moderately

viscous, and finally, a clear liquid suspension which gave a cooling effect on the skin (diagnostic indication for oil-in-water microemulsion). The clear oil-in-water emulsion was stable on storage.

5

Example 12

Transdermal Uptake Studies

10

A. Experimental protocol

The transdermal cell used for measuring skin penetration has upper and lower chambers which are separated by a skin patch. The lower chamber is designed to permit continuous flow through of saline, which collects drug penetrating from the outer side of the skin (exposed to the upper chamber) through the skin and into the saline in the lower chamber. An infusion pump is used to move through the chamber at a controlled rate (about 5 ml/hour).

20

Female hairless mice, strain HRS/hr, were obtained from Simonsen (Gilroy, CA). The animals were 7-8 weeks old, and weighed 20-30 gm when used. After sacrifice, three 2 cm diameter skin patches were removed from each animal. The patches were individually mounted in the cell, and held sealed against the lower chamber by an O-ring which is pressed against the patch by clamping.

25

Prior to adding the drug solution to the skin, a phosphate buffered saline solution was pumped through the system, at a flow rate of about 5 ml/hr for one hour. Fractions were collected continuously from the outlet side of the lower chamber, and dispensed into vials in a fraction collector. Collection time per fraction was one hour. Fractions were collected for up

30

-33-

to 24 hours after the drug solution was applied to the skin membrane. After the test period, the skin patch is washed several times, and removed. The hourly fractions, wash fractions obtained at the end of the experiment, and the skin patch itself were counted for radioactivity by conventional scintillation counting methods.

B. Control skin penetration test

The control vehicle was Rogaine™, obtained from Upjohn Co. This formulation contains 2% minoxidil in an ethanol/propylene glycol/water solvent vehicle, and was labeled with tritiated minoxidil before testing. One hundred fifty μ l samples were applied to skin patches and the uptake of minoxidil across the skin monitored as described. Typical results for a 24 hour test period are shown in Figure 5, where the control drug data is indicated by the open squares in the figure. As seen, the rate of uptake of the drug in the control formulation is substantially linear over the test period, and reaches a cumulative maximum, at the end of the test period, of about $30 \mu\text{g}/\text{cm}^2$, corresponding to about 0.5-1.0% of the total drug applied to the skin.

Example 13

Transdermal Penetration:

Laureth Sulfosuccinate Compositions

The 5% and 2% minoxidil/lauryl sulfosuccinate compositions prepared as in Examples 1 and 2, respectively, were tested for transdermal uptake, using the experimental methods described in Example 10. Three duplicate runs were made with each of the two

-34-

formulations, along with the control formulation (Example 12). The results, expressed in terms of cumulative μg drug uptake/ cm^2 of skin patch, are shown in Figure 5, where the data for the 2% composition is indicated by crosses, and for the 5% composition, by open diamonds. The open squares indicate uptake data for the control system, as indicated above.

It is seen that both sulfosuccinate compositions give greater drug transdermal penetration than the control drug formulation. The final cumulative doses correspond to about 0.5-1.0 for control drug, 2% for the 5% composition and 5-6% for the 2% composition.

Example 14

Transdermal Uptake: Crodafos Composition

A 1% minoxidil/Crodafos™ N3 acid was prepared as in Example 5, and tested for transdermal uptake using the experimental methods described in Example 12. These results are from four replicate cells, and are plotted along with the control formulation (Example 12). The results, expressed in μg drug penetration/ cm^2 skin, are shown in Figure 6, where the data for the 1% minoxidil in Crodafos™ N3 acid is indicated by crosses; open squares indicate the control formulation.

It is seen that the Crodafos™ formulation causes more than an order of magnitude increase in cumulative drug uptake as compared to the control formulation, even though the control formulation has twice the drug loading.

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Example 15Transdermal Uptake: Taurocholic Acid Composition

5 The 2% minoxidil/taurocholic acid compositions prepared as in Example 6 were tested for transdermal uptake using the experimental method described in Example 12. Four replicate cells were run and the data is plotted in Figure 5 as open triangles. The open squares indicate transdermal penetration by the control
10 formulation. It can be observed that although taurocholic acid has the same drug loading as the control formulation, less drug is put through the skin.

Example 16

15

Transdermal Uptake:Lauryl Sulfosuccinate Liposome Composition

The 2% and 5% minoxidil/lauryl sulfosuccinate liposome compositions prepared as in Example 7 were
20 similarly tested for transdermal delivery, with the results therein in Figure 7. The control formulation is denoted by open squares, the 2% liposome formulation by crosses, and the 5% liposome formulation by open diamonds. The 2% formulation delivers 6 times the
25 cumulative dose of the control formulation at 24 hours, and the 5% formulation delivers 13 times the cumulative dose of the control at 24 hours.

Example 17

30

Transdermal Uptake:Oleamido PEG-2 Sulfosuccinate Liposomes

A 2% minoxidil/oleamido PEG-2 sulfosuccinate liposome composition prepared as in Example 8 was tested

-36-

for transdermal delivery of minoxidil using the experimental protocol described in Example 12. The data are plotted in Figure 8 as open diamonds, and compared to the control formulation (open squares) and the 2% minoxidil lauryl sulfosuccinate liposomes (crosses).

The oleamide PEG-2 composition produces an approximately threefold increase in transdermal uptake over the lauryl sulfosuccinate liposome preparation. It is evident that changing the hemiester sulfosuccinate in the composition can change the rate of transdermal delivery of drug.

Example 18

15

Transdermal Uptake:

Cholesterol Sulfate Liposome Composition

The minoxidil/cholesterol sulfate/PC composition was prepared as in example 9 and assayed for transdermal drug delivery as in Example 12. The results are plotted as open triangles in Figure 8. As seen, this composition showed no transdermal penetration, even though the composition was saturated with drug (1% wt/vol).

25

Example 19

Transdermal Uptake: LysoPA/PA Liposome Composition

The minoxidil/lysoPA/PA in 30% Tween-0™ composition prepared as in Example 11 was tested for transdermal drug delivery as in Example 12. The data plotted as crosses in Figure 9. This composition is similar to the control formulation during the first 12 hours, and then increases substantially relative to the control formulation during the next twelve hour period.

Example 20Transdermal Uptake: PA Liposome Composition

5 The minoxidil/PA in Tween-0 composition was
prepared as in Example 11, and assayed for transdermal
drug penetration as in Example 12. These data are also
plotted as open diamonds in Figure 10. This composition
delivers one-third the drug of the control formulation
10 (open squares) and one-sixth that of the composition
containing lyso PA (crosses). The results indicate that
lyso PA and not PA or Tween-0™ is responsible for
facilitating transdermal delivery of the drug.

15 Although the invention has been described with
reference to particular methods of preparation, modes of
drug administration, and transdermal uptake
characteristics, it will be appreciated that various
modifications and changes in the methods and results can
20 be made or achieved within the scope of the invention.

25

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IT IS CLAIMED:

1. A non-crystalline minoxidil composition comprising minoxidil complexed with an amphipathic compound having a pK less than about 5 and containing a single lipophilic chain moiety and a polar head group moiety selected from a sulfate, sulfonate, phosphate and phosphonate group.
2. The composition of claim 1, wherein the molar ratio of amphipathic compound to minoxidil is at least about 1:1, and the pH is between about 4-6.
3. The composition of claim 1, wherein the amphipathic compound ester is selected from the group consisting of sulfosuccinic acid hemiesters, alkyl phosphonates, and alkyl phosphate esters.
4. The composition of claim 3, wherein the organic acid monoalkyl ester is an ethoxylated sulfosuccinic acid hemiester.
5. The composition of claim 1, which further includes phospholipids at a phospholipid to minoxidil molar ratio of between about 1:1 and 4:1.
6. The composition of claim 1, wherein the minoxidil and amphipathic compound are dispersed in non-crystalline form in a chlorofluorocarbon solvent.
7. The composition of claim 6, for use in forming a suspension of liposomes containing minoxidil in substantially monomolecular form, which further includes a phospholipid, at a phospholipid to minoxidil

molar ratio of between about 1:1 and 4:1, wherein the composition is injected into an aqueous medium under conditions which result in liposome formation in the aqueous medium.

5

8. The composition of claim 1, which further includes oil-phase and surfactant components and the composition is a microemulsion.

10

9. The composition of claim 1, for use in administering minoxidil topically in ointment form, which further includes at least about 60% by weight of water, and minoxidil is present at a weight concentration of between about 1-5%.

15

10. The composition of claim 9, which further includes phospholipids, at a phospholipid to minoxidil molar ratio of between about 1:1 and 4:1.

20

11. The composition of claim 9, which further includes oil-phase and surfactant components and the minoxidil is present in microemulsion form.

25

12. A method of applying minoxidil topically in a non-crystalline form which remains non-crystalline at least several hours after application to skin, comprising complexing the minoxidil with an amphipathic compound having a pK less than about 5 and containing a single lipophilic chain moiety and a polar head group moiety selected from a sulfate, sulfonate, phosphate and phosphonate group.

30

13. The method of claim 12, wherein the amphipathic compound is selected from the group

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consisting of sulfosuccinic acid hemiesters, alkyl phosphonates, and alkyl phosphate esters.

5 14. The method of claim 12, for use in
applying minoxidil in ointment form, which further
includes formulating the minoxidil and amphipathic
compound in an aqueous medium, to a final minoxidil
weight concentration of between 1-5%, and a final weight
concentration of water of at least about 60%

10

 15. The method of claim 12, for use in
administering minoxidil in spray form, which further
includes dispersing the minoxidil and amphipathic
compound in a fluorochlorocarbon solvent, and spraying
15 the resultant mixture from valved cannister containing
the mixture under pressure.

 16. The method of claim 12, which further
includes adding a phospholipid to the mixture, at a
20 phospholipid to minoxidil molar ratio of between about
1:1 and 4:1, to form a liposomal minoxidil composition.

 17. The method of claim 12, wherein said
complexing includes first forming a stable water-in-oil
25 microemulsion containing oil-phase, surfactant and
amphipathic compound components, then adding minoxidil
to the microemulsion to a final minoxidil concentration
of between about 2-7 percent by weight.

30

 18. The method of claim 17, which further
includes, after addition of minoxidil to the
water-in-oil microemulsion, adding water until an
oil-in-water microemulsion forms.

19. A non-crystalline minoxidil composition in which minoxidil is:

(a) present at a weight concentration of between about 1-5%;

5 (b) complexed with an amphipathic compound having a pK less than about 5, at an amphipath:minoxidil molar ratio of at least about 1:1;

(c) dispersed in substantially non-crystalline form in an aqueous or lipophilic solvent; and

10 (d) maintained in non-crystalline form for a period of at least several hours after application to the skin.

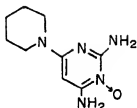
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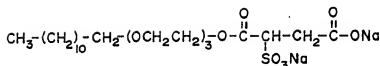
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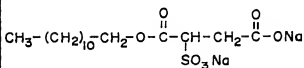
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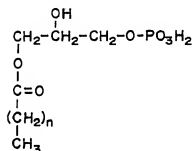
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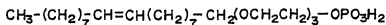
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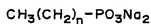
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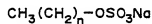
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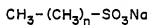
e. CRODAFO3 N3 ACID (OLETH 3 PHOSPHORIC ACID)



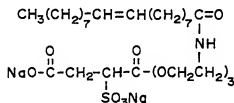
f. ALKYL PHOSPHONATE



g. ALKYL SULFATE ESTER



h. ALKYL SULFONATE



i. OLEAMIDO POLYETHYLENE

j. GYCOL-2 SULFOSUCCINATE HEMI-ESTER

FIG. 1

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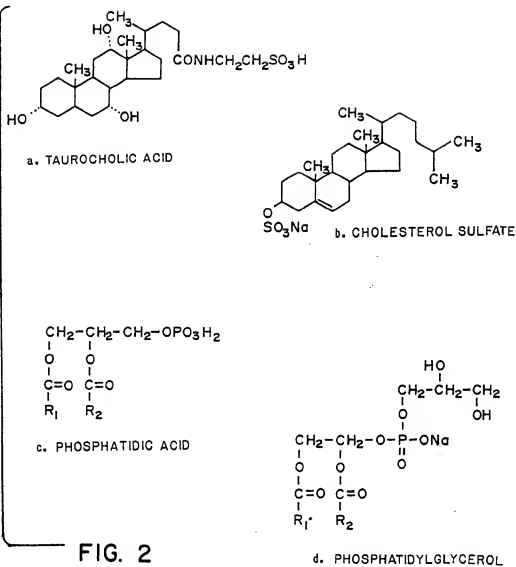
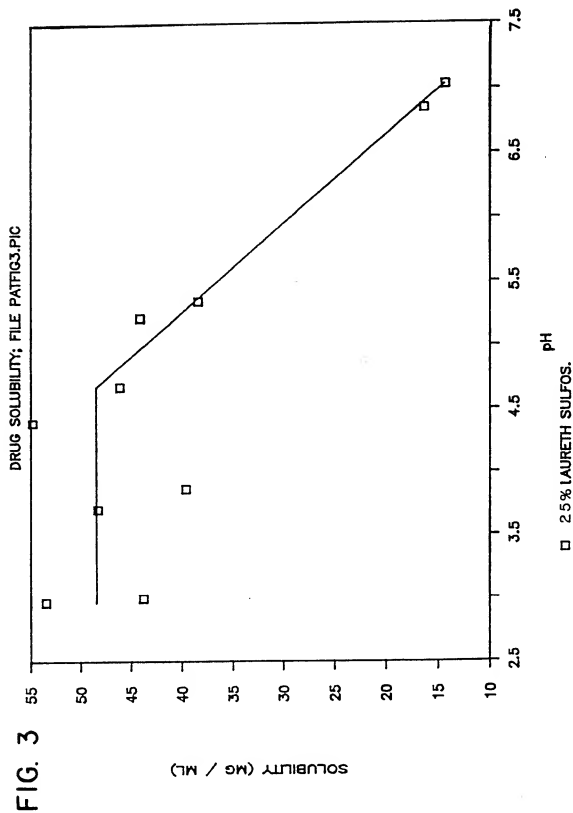


FIG. 2

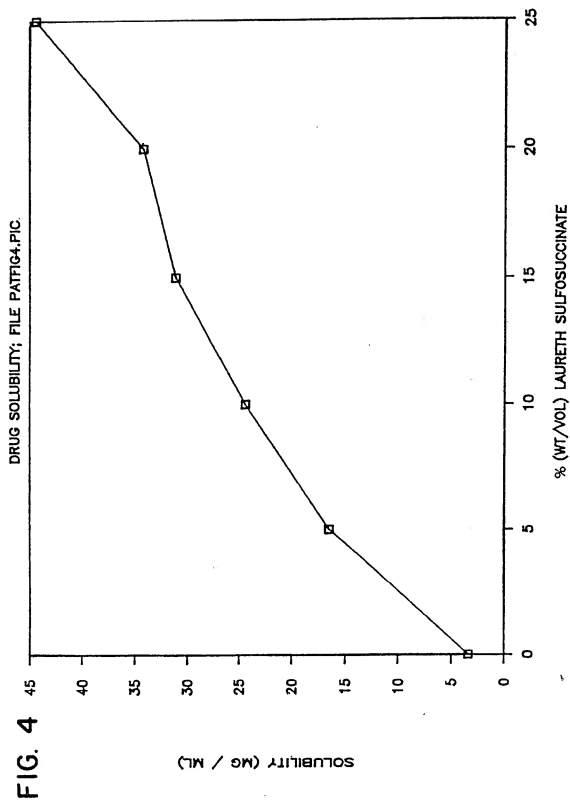
WHERE R_1 AND R_2 REPRESENT ANY FATTY ACID.

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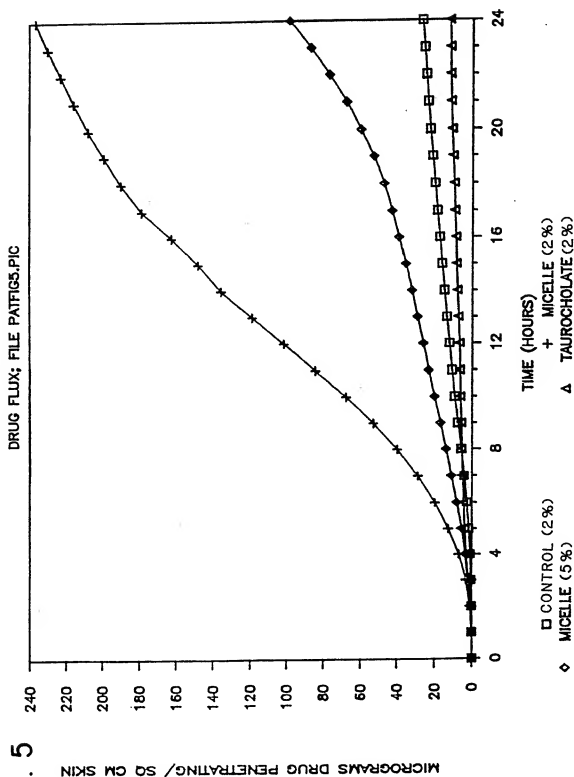


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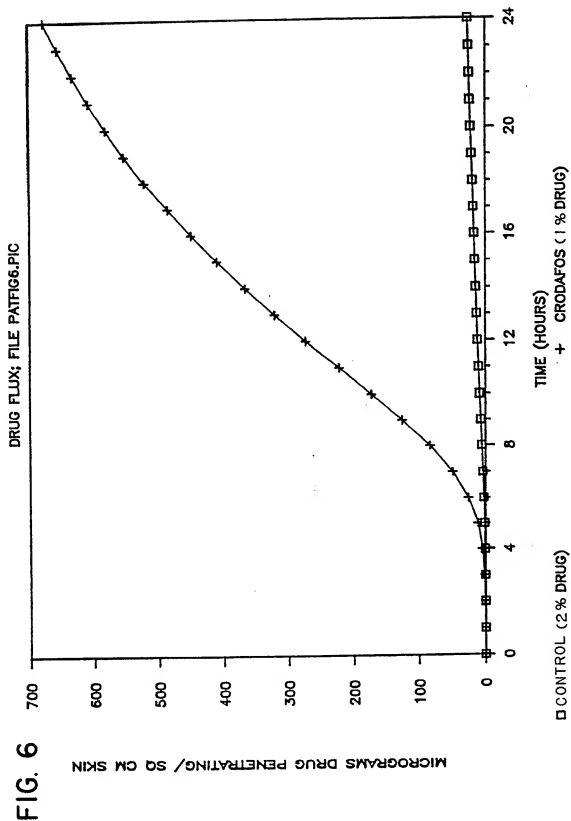
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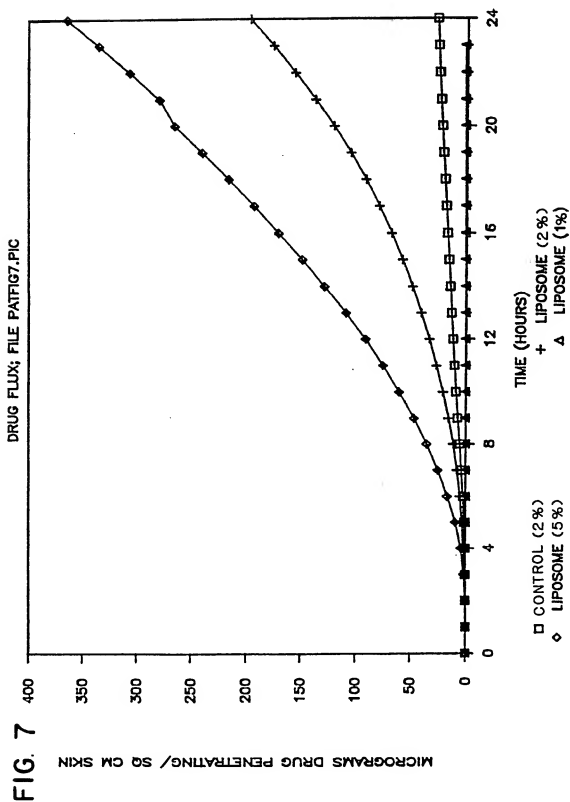


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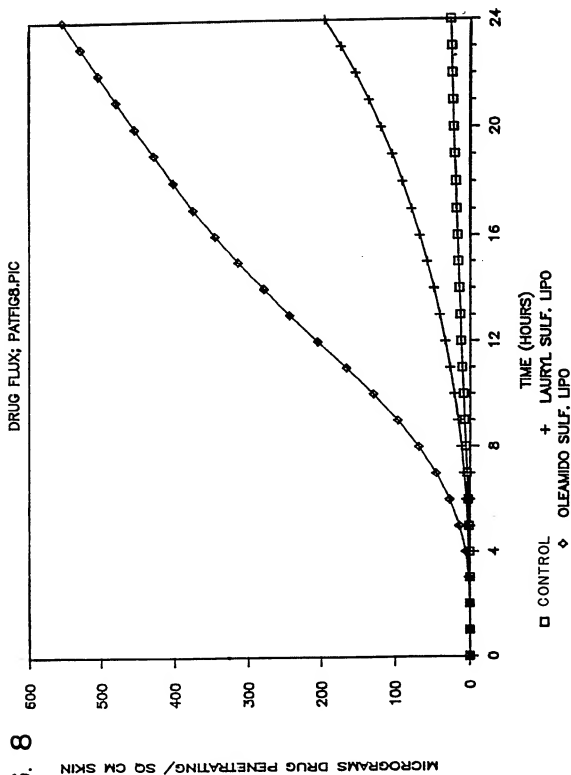
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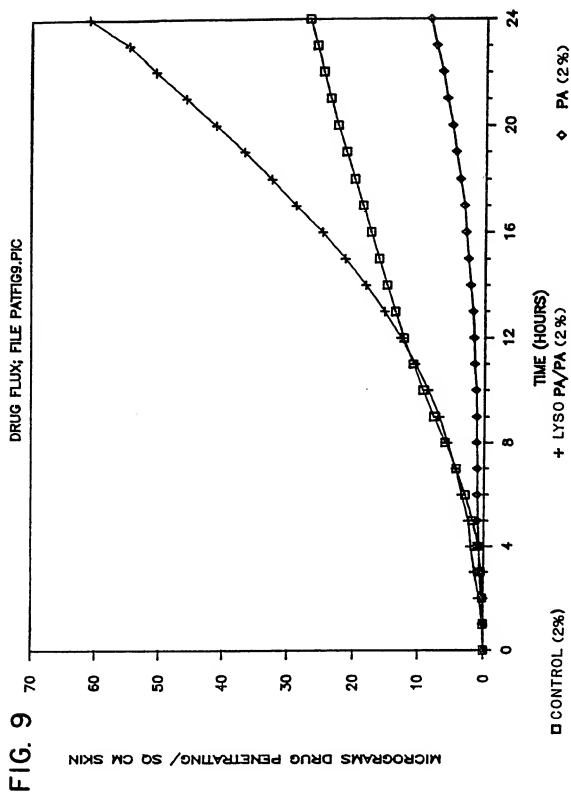
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INTERNATIONAL SEARCH REPORT

International Application No. **PCT/US88/00995**

I. CLASSIFICATION OF SUBJECT MATTER (In several classification symbols apply, indicate all):		
According to International Patent Classification (IPC) or to both National Classification and IPC INT. CL. 4 A61K 7/06, 9/66, 31/685, 37/22; B01J 13/02. U.S. CL. 264/4.1; 424/45, 450; 428/402.2; 436/829; 514/78, 947, 969.		
II. FIELDS-SEARCHED		
Minimum Documentation Searched *		
Classification System :	Classification Symbols	
 U.S. CL. 264/4.1; 424/45, 450; 428/402.2; 436/829; 514/78, 947, 969.		
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched *		
 III. DOCUMENTS CONSIDERED TO BE RELEVANT ^{1,4}		
Category *	Citation of Document, ^{1,6} with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
Y	EP, A, 0, 161, 445 PUBLISHED 21 NOVEMBER 1985 GREEN CROSS CORP. SEE ABSTRACT; PAGE 3, LINE 16-PAGE 4, LINE 2; PAGE 7, LINE 14-PAGE 8, LINE 11; AND EXAMPLES.	1-3, 5-19
Y,P	US, A, 4,654, 354 PUBLISHED 31 MARCH 1987 SHROOT ET AL SEE COL. 5, LINES 26-41; COL. 6, LINES 3-9 AND 55-57; AND COL. 7, LINES 5-12.	1-3, 5-19
Y	GB, A, 2, 145, 107 PUBLISHED 20 MARCH 1985 MCGURK ET AL. SEE PAGE 1, LINE 50-PAGE 2, LINE 4; PAGE 2, LINES 18-35 AND 43-58; EX. 1; AND ABSTRACT.	6-8, 11, 15, 17, 18
A,P	US, A, 4, 670, 185 PUBLISHED 02 JUNE 1987 FUJIWARA ET AL.	1-19
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Date of the Actual Completion of the International Search :		Date of Mailing of this International Search Report :
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ISA/US		<i>Richard D. Lovering</i> RICHARD D. LOVERING



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US90/00918 (22) International Filing Date: 22 February 1990 (22.02.90) (30) Priority data: 315,392 24 February 1989 (24.02.89) US 356,262 23 May 1989 (23.05.89) US (71) Applicant: LIPOSOME TECHNOLOGY, INC. [US/US]; 1050 Hamilton Court, Menlo Park, CA 94025 (US). (72) Inventors: USTER, Paul, Steven ; 883 Warren Way, Palo Alto, CA 94303 (US). FIELDING, Robert, M. ; 1806 Stockbridge, Redwood City, CA 94061 (US). MORANO, Jacqueline, K. ; 2124 Rock Street 34, Mountain View, CA 94043 (US). MARTIN, Francis, J. ; 415 West Portal Avenue, San Francisco, CA 94127 (US).		(74) Agent: DEHLINGER, Peter; The Law Offices of Peter J. Dehlinger, 350 Cambridge Avenue, Suite 100, Palo Alto, CA 94306 (US). (81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: LIPOSOME GEL COMPOSITION AND METHOD		
(57) Abstract A gel-like liposome composition and method of preparing the same. The composition is composed of charged liposomes, at a relatively low lipid concentration, in a low-conductivity medium. The composition preferably contains a zwitterionic species at its isoelectric point. The liposomes can be designed for cosmetic use, transdermal drug delivery, or enhanced retention on mucosal tissues, such as for ophthalmic use. In one embodiment, the gel composition contains encapsulated and/or surface bound epidermal growth factor, for use particularly in surgical wound healing.		

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LIPOSOME GEL COMPOSITION AND METHOD1. Field of the Invention

- 10 The present invention relates to a high-viscosity liposome gel composition, and to methods of making and using the composition.

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20

3. Background of the Invention

- Lipid bilayer vesicles, or liposomes, have been proposed for use in a variety of topical applications. In the cosmetics industry, liposome formulations are
- 25 currently sold as a lipid supplement to enhance dry or aging skin. Liposomes may also be useful for applying compounds, such as ultraviolet-blocking agents, vitamin A, retin, and the like to the skin, to achieve greater drug solubility or skin compatibility, reduced irritation
- 30 from the drug, and/or extended drug release.

Liposomes also offer the potential of improved transdermal drug delivery. It is known, for example, that liposomes are able to facilitate the uptake of

certain lipophilic compounds, such as anti-inflammatory steroid compounds, across the skin barrier (Mezei, 1982, 1983), and the drug-uptake characteristics of the liposomes can be modulated by varying lipid composition in the liposomes. Additionally, the liposomes can be formulated with coentrapped agents, such as azacycloalkane-2-ones, which facilitate transdermal uptake of drugs (U.S. Patent No. 4,316,893), to improve and/or modulate transdermal drug release characteristics.

10 Liposomes are also promising drug-delivery vehicles for sustained drug release on mucosal surfaces, including corneal tissue. In ophthalmic use, for example, liposomes can provide delayed drug release, and greater solubility of lipid-soluble drugs, for release at the
15 corneal surface, and liposomes alone are useful as a lipid supplement for dry eye (U.S. Patent No. 4,818,537). Additionally, liposomes can be engineered for enhanced retention on mucosal surfaces, to extend the period of effective drug delivery with each liposome application
20 (U.S. Patent No. 4,804,539).

 In all of the above topical uses of liposomes, it is generally desirable to administer the liposome preparation in a viscous form. In particular, the ideal liposome preparation is a gel which is preferably sufficiently
25 viscous to give persistence at the site of application, especially at a wound site or mucosal tissue site. In cosmetic applications, the gel material should be clear or translucent and preferably be non-greasy to the touch.

30 Heretofore, viscous liposome pastes have been prepared by forming liposomes at high lipid concentrations, for example, by concentrating dilute liposome preparations. The high lipid concentrations make these formula-

ctions relatively expensive. The viscosity of the paste material may also complicate processing steps used for example, to sterilize the liposomes or remove non-entrapped drug molecules. Further, liposome paste preparations are generally greasy to the touch.

Liposome gel formulations have been produced heretofore by suspending liposomes in gel-forming colloidal materials, such as Hydrogel™, collagen, synthetic polymers, and the like. Although liposome-in-gel formulations of this type can be prepared with desired physical properties, the gel-forming matrix itself may be toxic or otherwise incompatible with the site of application.

4. Summary of the Invention

It is therefore one object of the invention to provide a high-viscosity liposome gel composition which provides many of the above-discussed desired features of a viscous liposome formulation for topical use.

It is a more specific object of the invention to provide a high-viscosity EGF/liposome gel composition which can be applied to a wound or surgical incision, for retention and sustained release of EGF at the site of application.

It is another object of the invention to provide a method for treating a wound or incision with such composition.

The invention includes, in one aspect, a high-viscosity liposome gel composition for use in topical application to the skin, in skin wounds, and on mucosal tissue. The composition includes a suspension of charged liposomes in a low-conductivity aqueous suspension medium which has a selected pH between about 5.5 and 8.5. The charged liposomes contain between about 5-50 weight

percent charged vesicle-forming lipids, and the balance of neutral vesicle-forming lipids. The concentration of lipids in the composition is between about 7-25 weight percent and preferably between about 8-12 percent.

- 5 The aqueous suspension preferably contains a zwitterionic compound, such as a neutral amino acid, whose isoelectric point is at the selected pH of between about 5.5 and 8.5.

- 10 In one general embodiment, the charged vesicle-forming lipids include negatively charged lipids phospholipid components, such as phosphatidylglycerol (PG). One preferred liposome composition includes approximately equal weight proportions of PG, phosphatidylcholine (PC), and cholesterol.

- 15 The EGF/liposome composition of the invention includes a high-viscosity suspension of negatively charged EGF/liposomes, i.e., liposomes containing EGF in liposome-entrapped form. The EGF/liposomes contain neutral phospholipid, and at least about 10 weight percent negatively charged phospholipid, and preferably, between 20-
20 50 weight percent each of neutral phospholipid, negatively charged phospholipid, and cholesterol. The total lipid concentration of the EGF/liposomes in the composition is at least 50 mg/g composition and preferably
25 between 50-200 mg/g composition. The EGF may be entrapped in the EGF/liposomes by encapsulation or surface adsorption or a combination of both.

- 30 In another general embodiment, for use in administering a drug to mucosal tissue, the charged vesicle-forming lipids include positively charged lipid components, such as a phosphatidylethanolamine conjugate prepared by derivatizing phosphatidylethanolamine with a basic amino

acid, or a benzylamine lipid, such as benzyldimethylstearylammmonium chloride (BDSA).

The liposome gel composition is formed, according to the method of the invention, by adding a mixture of vesicle-forming lipids containing between about 10-50 weight percent components having a common charge at a selected pH between about 5.5 and 8.5, with a low-conductivity aqueous suspension medium, at a final total lipid concentration of between about 7-25 weight percent.

The lipids may be added directly to a low-conductivity aqueous medium or, alternatively, to an aqueous medium containing a zwitterionic compound whose isoelectric point is substantially different from that of the pH of the medium, such that the medium is not characterized by low conductivity. Following formation of a fluidic liposome suspension, the medium is titrated to a pH at which the zwitterionic compound is at its isoelectric point, yielding a low-conductivity condition which produces gel formation in the suspension. The liposome suspension may be more easily sized, freed of non-liposome-bound drug, filter-sterilized or otherwise processed in the more fluidic state prior to gelling.

These and other objects and features of the invention will become more fully apparent when the following detailed description of the invention is read in conjunction with the accompanying drawings.

Brief Description of the Drawings

Figure 1 is a plot of viscosity of a liposome suspension formed in accordance with the present invention, as a function of concentration of univalent electrolyte;

Figures 2A-2C show the electrical potential seen by charged particles as a function of distance from the

surface of a negatively charged liposome for high lipid concentration and high inner strength (2A), low lipid concentration and high inner strength (2B), and low lipid concentration and low inner strength (2C);

5 Figure 3 illustrates the change in calculated Debye length as a function of univalent electrolyte for charged liposomes having lower (solid line) and higher (dashed line) surface charge densities;

10 Figures 4 and 5 are Scatchard plots of EGF binding to EPG/EPC and EPG/EPC/cholesterol liposomes, respectively;

15 Figures 6 and 7 are plots of surface pressure, at an air/water interface, of aqueous EGF (Figure 6) and EPG/-EPC/cholesterol (Figure 4), respectively, as a function of EGF and liposome concentration;

 Figure 8 is a plot of change in surface pressure, as a function of initial surface pressure, in the presence and absence of EGF in EPC/EPG/cholesterol liposomes (open triangles) and PC/PG liposomes (solid circles);

20 Figures 9-12 show the change in free EGF available in the donor compartment of a two compartment flux chamber, plotted as a function of time for free EGF (Figure 9) and for three EGF/liposome compositions (Figures 10-12);

25 Figures 13-16 show the retention of radiolabelled EGF, plotted as a function of time, for free EGF (Figure 13), and for three EGF/liposome compositions (Figures 14-16);

 Figure 17A-17C illustrate surgical steps in a corneal implant operation; and

30 Figures 18A-18C are diagrammatic cross-sections of the surgical region of an eye seen in Figures 17A-17C, showing in Figure 18B the introduction of an EGF/liposome formulation prepared according to the invention, and in

Figure 18C, the residual composition in the eye after an extended release period.

Detailed Description of the Invention

5

I. Liposome Gel Compositions

This section describes components and methods used in forming the high-viscosity liposome composition of the invention.

10 A. Definitions

As used herein, the terms below have the following meaning:

1. "Neutral vesicle-forming lipids" refers to any lipid or lipid mixture (i) capable of forming stable
15 lipid bilayer vesicles in the presence of charged vesicle-forming lipids, at a selected concentration of charged lipids between 5-50 weight percent of total lipids, and (ii) having a polar head group with no net charge at a pH between about 5.5-8.5.
- 20 2. "Charged vesicle-forming lipids" refers to any amphipathic lipid (i) capable, at a selected concentration between 5-50 weight percent, of forming stable lipid bilayers in the presence of neutral vesicle-forming lipids, and (ii) having a polar head group with a net
25 negative or positive charge at a pH between about 5.5-8.5.
3. "Negatively charged phospholipid" refers to any vesicle-forming lipid having (i) two hydrocarbon-chain
30 moieties which are effective to produce a stable bilayer formation, and (ii) a polar head group with a net negative charge at a pH between about 5.5-8.5.

4. "Cholesterol" refers to cholesterol or any related sterol capable of combining with phospholipids to form stable lipid-bilayer vesicles.

5. "Epidermal Growth Factor" or "EGF" refers to human-EGF (h-EGF), typically recombinantly produced human EGF (rh-EGF), and to related peptides having the requisite ability to promote the growth of a variety of cells of epithelial origin in vitro.

6. "High-viscosity" or "gel" or "gel-like" refers to a viscous, relatively non-flowable gel consistency which can be applied by squeezing from a tube or syringe, but which is sufficiently non-flowable, once applied, to be retained in bolus form at a wound or incision site for at least several hours.

7. A "low-conductivity aqueous medium" refers to an aqueous medium whose conductivity is no more than that of a fully ionized univalent electrolyte whose concentration is between about 5-10 mM. Typically, the low-conductivity medium is one which reduces the Debye length of a charged liposome by no more than half its value at a concentration of fully ionized univalent electrolyte of about 1 mM.

B. Lipid Components

The liposome gel composition formed in accordance with the invention is prepared to contain between about 50-95 weight percent neutral vesicle-forming lipids, and about 5-50 weight percent charged vesicle-forming lipids which impart a net negative or net positive charge to the liposome surfaces.

Preferred neutral vesicle-forming lipids are phospholipids, such as phosphatidylcholine (PC), and cholesterol. Neutral phospholipids lipids having a

variety of acyl chain groups of varying chain length and degree of saturation are available, or may be isolated or synthesized by well-known techniques. In general, partially unsaturated phosphatidylcholine (PC), such as egg PC (EPC) or soy PC (SPC), or fully or partially hydrogenated egg PC (HEPC) or soy PC (HSPC) are readily obtained and provide suitable liposome characteristics, such as ease of extrusion and stability.

Cholesterol and related uncharged neutral analogues thereof, such as 5,6,-cholestene and cholestane, are typically present at about 20-50 weight percent. Cholesterol is known to increase the stability of liposomes and, in the case where the phospholipid components are relatively unsaturated, to increase the packing density of the lipids in the liposomal bilayers. One advantage of cholesterol, where the liposomes are applied at a wound or surgical site, is potentially reduced toxicity due to lipid exchange between the liposomes and cells at the wound or surgical site. It has been demonstrated, for example, with several cultured tumor cell lines, that liposomes containing entrapped epidermal growth factor (EGF) inhibit cellular growth in vitro, and that for at least some cell lines, this inhibition can be greatly reduced by the addition of cholesterol to EPC liposomes (Mayhew).

Preferred negatively charged vesicle-forming lipids include negatively charged phospholipids, such as the negatively charged phospholipids phosphatidylglycerol (PG), phosphatidylserine (PS), and phosphatidylinositol (PI). One preferred negatively charged phospholipid is partially or fully saturated PG, such as egg PG (EPG). Alternatively, or in addition, the charged components may

preferably include charged cholesterol derivatives such as cholesterol sulfate and cholesterol hemisuccinate.

One preferred type of positively charged vesicle-forming lipids include positively charged phospholipids, such as phosphatidylethanolamine (PE) or phospholipids which have been derivatized at their polar head groups with amines, to produce a net positive charge. Methods of producing derivatized phospholipids of this type are described in co-owned U.S. Patent No. 4,804,539. By way of example, PE can be derivatized with basic amino acids, such as lysine, to produce vesicle-forming lipids whose positive charge is separated from the phosphate groups of the lipid by a several-atom spacer.

Another general class of positively charged vesicle-forming lipids include benzyl/aliphatic-chain amines which are (a) capable of being anchored in a liposome bilayer by virtue of the aliphatic chain, and (b) carry a net positive charge at a selected pH between about 5.5 and 8.5. The aliphatic chain is at least about 12 carbons in length, and the amine is preferably a quaternary amine whose remaining (two) nitrogen linked groups are short-chain alkyl groups, such as methyl or ethyl groups, as detailed in U.S. Patent No. 4,818,537. One preferred compound is benzyltrimethylstearylammmonium chloride (BDSA).

Cholesterol amines form another class of positively-charged vesicle-forming lipids which are suitable for use in the invention. Cholesterol derivatives of the type Ch-O-C-Y-N and Ch-NH-Y-N , where ChOH is cholesterol, and Y is a short carbon-containing chain, have been described.

Finally, the charged lipid component may include a lipophilic drug which tends to be tightly bound to the lipid bilayer phase of the liposomes.

- The lipid components forming the liposomes contain
- 5 at least about 5-10 weight percent charged lipid component, and preferably between about 20-40 weight percent charged lipid. The balance of the lipids are neutral vesicle-forming lipids. The following lipid compositions, expressed in weight percent, are exemplary:
- 10 1. HEPC:EPG, 95:5;
2. EPC:EPG, 80:20;
3. EPC:EPG, 50:50;
4. EPC:EPG:cholesterol, 50:20:30;
5. EPC:EPG:cholesterol, 33:33:33;
15 6. EPC:cholesterol sulfate, 80:20;
7. EPC:cholesterol:cholesterol sulfate 50:30:20;
8. FSFC:PE 80:20;
9. FSFC:lysiny PE 80:20;
10. EPC:cholesterol:cholesterol amine 60:20:20;
20 11. PC:BDSA 90:10; and
12. PC:BDSA 75:25.

- It is noted that the total amount of neutral and charged cholesterol together is preferably no more than 50 weight percent. Further, it is understood that the
- 25 liposome composition may contain a variety of other lipid components which may enhance liposome stability, viscosity, or drug release characteristics, and/or materials cost. For example, the liposomes may include α -tocopherol, or pharmaceutically acceptable analogue thereof, at
- 30 a total concentration of between about 0.1 to 2 weight percent, to improve lipid stability on storage.

EGF/liposomes formed in accordance with the invention are prepared to contain between 10-90 weight percent

neutral phospholipid, and 10-50 weight percent negatively charged phospholipid, and preferably between about 20-50 weight percent each of neutral phospholipid, negatively charged phospholipid, and cholesterol.

- 5 The negatively charged phospholipid in the composition serves two important roles. First, it imparts a negative charge to the lipid bilayer membranes, providing an electrostatic interaction between the membrane and the positively-charged EGF. The adsorption of EGF to the
- 10 liposomal membrane will be discussed below. Secondly, the relatively high surface charge is important in the formation of a gel-like liposome state which is characterized by a low lipid concentration and high viscosity, as described below.
- 15 The effect of cholesterol on the rate of EGF release from EGF liposomes has been examined both in vitro and in vivo, as detailed below. Briefly, cholesterol significantly increased the half-life of EGF release in vivo. Another advantage of cholesterol in the EGF/liposome
- 20 composition is potentially reduced toxicity due to lipid exchange between the liposomes and cells at the wound or surgical site, as noted above.

C. Low-Conductivity Aqueous Medium

- 25 According to an important feature of the invention, it has been discovered that hydration of vesicle-forming lipids having the above composition with a low-conductivity aqueous medium produces a liposome composition which is both gel-like in consistency and viscosity, and
- 30 has a relatively low lipid concentration.

More specifically, the combination of surface charge on the liposomes, due to the presence of charged lipid component(s) and the low-conductivity aqueous medium

produces a liposome composition characterized by (a) a viscous, gel-like consistency and (b) a relatively low lipid concentration, e.g., 50-250 mg/g composition and 7-25 weight percent lipid.

5 The aqueous medium preferably includes a zwitterionic compound whose isoelectric point (at which the compound is effectively a non-electrolyte) is at the selected pH of the medium between pH 5.5 and 8.5. Neutral amino acids, such as glycine, isoleucine alanine, pro-
10 line, and valine are preferred zwitterionic compounds. The final concentration of zwitterionic compound in the buffer is typically at least about 0.5 percent by weight and preferably between about 1-5 percent by weight, and the buffer is adjusted in pH to the isoelectric point of
15 the compound to achieve the gel state.

As will be discussed in Section D below, the aqueous medium may initially be adjusted to a pH at which the zwitterionic compound is substantially in a charged form, so that the medium has a relatively high electrolyte
20 concentration, i.e., a relatively high conductivity. By adjusting the pH to the isoelectric point of the zwitterionic compound, typically after lipid hydration and liposome formation, the compound becomes non-electrolytic, i.e., has the desired low conductivity. It is
25 noted, however, that the final salt concentration of the medium, after adjusting the pH to the isoelectric point of the zwitterionic compound, must not produce a significant increase in the ionic strength of the medium. This objective can be achieved, for example, by employing
30 volatile ammonium salts, or as described below, by employing an initial low concentration of zwitterionic compound.

The zwitterionic compound is preferably present at a concentration of between about 1-3 weight percent aqueous medium, and preferably at a concentration which renders the final composition substantially isotonic. Alternatively, or in addition, the aqueous medium may include other non-electrolyte solute compounds, such as sugars, uncharged water-soluble drugs, and the like which produce a desired osmolarity of the final gel composition.

10 D. Preparing the Gel Composition

The gel composition of the invention is formed by mixing the neutral and charged vesicle-forming lipids described in Section B with a low-conductivity aqueous medium, at a final lipid concentration of between about 15 7-25 weight percent lipid, and preferably between about 10-15 weight percent lipid.

In one general embodiment of the method, the lipids are added directly to the low-conductivity medium, such that when the selected final lipid concentration is 20 reached, the suspension assumes a gel-like state at room temperature.

In one procedure, vesicle-forming lipids are dissolved in a suitable organic solvent or solvent system and dried under vacuum or an inert gas to form a thin 25 lipid film. If desired, the film may be redissolved in a suitable solvent, such as tertiary butanol, and then lyophilized to form a more homogeneous lipid mixture which is in a more easily hydrated powder-like form.

This film is covered with a selected amount of the 30 low-conductivity medium, and allowed to hydrate, typically over a 15-60 minute period with agitation. The size distribution of the resulting multi-lamellar vesicles (MLVs) can be shifted toward smaller sizes by hydrating

the lipids under more vigorous agitation conditions. The final concentration of liposomes is at least 70 mg/g and preferably between about 100-150 mg/g composition. The hydration step is generally effective to produce a homogeneously hydrated liposome suspension, where relatively small lipid quantities are involved.

For larger lipid amounts, the hydrated suspension may contain particles of non-hydrated or partially hydrated lipids. This suspension can be converted to a homogeneous suspension by further processing, preferably by extrusion through a defined-pore size membrane, such as a 2 micron pore size polycarbonate membrane. The extrusion step, of course, also reduces the size heterogeneity in the suspension. This general procedure for preparing a liposome gel suspension is illustrated in Example 1.

In another procedure, the lipids are added to the low-conductivity medium by injecting the lipids in a lipid-in-solvent solution into the medium, until the desired lipid concentration (gel viscosity) is reached. This method is illustrated in Example 7.

It will be appreciated that water-soluble drugs or agents can be encapsulated in the liposomes formed in the gel by dissolving the drug or agent in the hydration medium. Similarly, a lipophilic compound can be conveniently added to the lipid mixture prior to hydration, for preparing liposomes with entrapped lipophilic drug.

In a second general embodiment of the method, the lipids are added to an aqueous medium containing a zwitterionic compound, at a pH which is substantially different from the isoelectric point of the compound. In particular, the concentration of zwitterionic molecules having a net positive or negative charge is such that the aqueous medium cannot be characterized by low conduc-

tivity. Typically the medium contains at least about 20 mM zwitterionic compound having a net positive or negative charge. For example, the medium may be 100 mM zwitterionic compound, at a pH at which 20 percent of the compound has a net charge.

The liposome suspension formed in the aqueous medium is relatively fluidic, or non-viscous, being characterized by high flow characteristics. Because of its low viscosity, this suspension is easily processed to achieve desired liposome/suspension characteristics. For example, the suspension may be further processed to (a) achieve smaller and or more uniform liposome sizes, (b) remove free water-soluble drug and/or (c) sterile the EGF/liposome preparation.

A variety of techniques are available for reducing liposomes to a desired size range, including sonication, homogenization and extrusion through a defined-pore size membrane. Extrusion of liposome through a small-pore polycarbonate membrane has been used successfully, as has extrusion through asymmetric ceramic membranes (U.S. Patent No. 4,737,323). The liposomes may be extruded through successively smaller-pore membranes, to achieve a gradual reduction in liposome size.

Free water-soluble drug can be removed, if desired, by conventional centrifugation, ultrafiltration, or gel filtration (molecular sieve) methods. When the liposomes are sized by extrusion, free drug is preferably removed following the extrusion step.

The liposome suspension may be sterilized, after sizing, by filtration through a conventional depth filter, typically having a 0.22 micron particle exclusion size.

After liposome processing, the non-viscous liposome suspension is converted to the desired gel form by titrating the pH of the suspension to a isoelectric point of the zwitterionic species. As mentioned above, the titration must be carried without significantly increasing the concentration of dissociable salts in the medium. This can be done by titrating with acids or bases which produce volatile salt components, such as certain ammonium salts, or which produce insoluble salts. Preferably, the titration is done by forming an initial liposome suspension in a medium containing low zwitterionic concentration, then titrating with a concentrated solution of the same zwitterionic compound, until the desired pH is reached.

It will be appreciated that a variety of liposome preparation methods, including reverse-phase evaporation and solvent-injection methods (Szoka, 1978, 1980), can be adapted for preparation of a liposome gel composition, using a low ionic-strength aqueous medium in the liposome formation step, in accordance with the invention.

The EGF/liposome gel composition of the invention can be prepared conveniently by a modified thin-film hydration method, as described above. Briefly, a lipid thin film is covered with hydration medium and allowed to hydrate, typically over a 15-60 minute period with agitation, with a low-conductivity buffer, as above. The final concentration of EGF/liposomes is at least 50 mg/g and preferably between about 50-200 mg/g composition.

This method is illustrated in Example 1.

Alternatively, the liposome gel composition can be formed in two stages, involving initial liposome formation of a fluidic liposome suspension, by addition of the

aqueous buffer in an electrolytic condition (e.g., where the zwitterionic compound is not at its isoelectric point, and subsequent pH adjustment of the aqueous medium to a non-electrolytic state (isoelectric point of the medium), to produce the desired gel formation. The intermediate non-viscous liposome composition can be processed, as above, e.g., to achieve a desired liposome size range. This method is illustrated in Example 3.

The aqueous medium used in forming the composition may contain dissolved EGF, at a suitable concentration. The suspension formed in this manner includes encapsulated, liposome-adsorbed, and free EGF. Free EGF can be removed, if desired, by conventional methods, such as molecular sieve filtration or the like.

Alternatively, free EGF may be added to preformed liposomes at a suitable concentration, producing a suspension with liposome-adsorbed and free EGF. According to one aspect of the invention, it has been found that the in vivo release kinetics of EGF from EGF/liposomes containing adsorbed EGF only is comparable for EGF/liposomes prepared to include both adsorbed and encapsulated EGF (Example 5).

From the foregoing, several advantages of the method of preparation of the liposome gel composition of the invention can be appreciated. The gel composition can be prepared at a low lipid concentration and thus is relatively inexpensive to manufacture. The final viscosity of the composition can be controlled by small changes in final ionic strength, produced either by addition or removal of ionic components, or by relatively small pH changes in a medium containing a zwitterionic buffer.

The liposome gel can easily be prepared and processed in a dilute form, for example to remove free drug, such as EGF, and to size and sterilize the liposomes, then brought to a final viscous state by pH adjustment.

- 5 Finally, as discussed in below, for preparation of an EGF\liposome gel composition, the EGF is adsorbed readily to the negatively charged liposomes in the EGF/-liposome composition, allowing the composition to be prepared simply by mixing free EGF with preformed liposomes.
- 10

E. Viscosity of the Gel Composition

- The liposome gel composition of the invention is characterized by a high-viscosity gel-like consistency which is maintained at a low ionic strength, but which collapses as ionic strength is increased. This feature is illustrated in the study described in Example 2. Here liposomes containing equal-weight amounts of EPG, EPC, and cholesterol were prepared in a 2.3% w/v glycine buffer at isotonic pH (pH 6.0) buffer, as detailed in Example 1.
- 15
- 20

- The mean viscosity for the samples was 13.3×10^3 Cps (centipoise) at 1.0 per second shear rate, characterized by a thick, relatively non-flowing gel consistency. With addition of NaCl to a concentration of only 0.05% w/v (about 8.5 mM), the material lost its gel-like properties, being quite fluid, with a mean viscosity of only about 2.7×10^3 Cps at 1 per second. Further relatively small decreases in viscosity were seen with further addition of NaCl to a final concentration of 0.2% w/v. The loss of viscosity at low NaCl concentration is seen in Figure 1.
- 25
- 30

The nature of the high viscosity gel composition can be appreciated from the liposome suspension models illustrated in Figures 2A-2C. The model shown in Figure 2A represents a liposome paste or concentrate containing a maximum concentration of lipid vesicles in an aqueous suspension medium. Empirically, viscous, paste-like lipid suspensions having a lipid concentrations of up to about 500 µg/ml can be produced, at which about 70% of the total aqueous volume is encapsulated.

The model of a liposome concentrate shown in Figure 2A assumes that at high lipid concentrations, and in the absence of surface charge effects, liposomes are able to form close packed suspensions in which the liposomes are densely packed, as indicated, being separated from one another only by a thin shell of ordered water (shown in dotted line). This model is consistent with the high percent of encapsulated water (up to 70%) observed in high-concentration liposome paste formulations.

Figure 2B shows a conventional liposome suspension containing about one-fifth the lipid concentration, e.g., 100 µg/ml. Assuming the suspension is composed of the same types of liposomes, more than 80% of the aqueous medium in the suspension would be non-encapsulated water, and each liposome would now be free to move through relatively large aqueous volume elements, as indicated. Because of this liposome mobility the suspension has a very low viscosity, i.e., is freely flowable.

Figure 2C shows the same low concentration of liposomes as in Figure 2B, but in a suspension formed in accordance with the invention in which the liposomes contain at least about 7 weight percent charged lipid component and a low-conductivity medium. The low lipid concentration of the suspension indicates that more than

80% of the total volume of the suspension is extra-liposomal water, i.e., non-encapsulated water. However, the high viscosity of the medium indicates that the liposomes are arrayed in packed spheres, as illustrated in Figure 2A.

These two assumptions are consistent with a model in which each liposome is surrounded by a relatively large spherical shell which contains a volume of up to several times that of the liposome, but which itself cannot be readily penetrated by the shells of neighboring liposomes. The thickness of the spherical water shells can be approximated from the following simplifying assumptions: (a) the maximum volume of liposome-encapsulated medium at a lipid concentration of 500 $\mu\text{g/ml}$ is 70 percent; (b) in both high-and low-concentration suspensions, the uniform liposome sizes of about 2,000 \AA ; and (c) the total number of liposomes which is proportional to lipid concentration. The shell thicknesses given in Table 1 below can be calculated:

20

Table 1

	Lipid Concentration	Shell Thickness
	($\mu\text{g/ml}$)	(\AA)
25	100	1400
	200	700
	300	400
	400	150
	500	0

30

Since the immobilization of the liposomes in the dilute suspensions is assumed to be due to charge repulsion among charged, unshielded particles, the thicknesses of these

shells provide a rough estimate of the distance over which the charged liposomes exert an appreciable charge repulsion effect.

The concept of an electrostatic liposome shell is analogous to Debye length, which corresponds roughly to the distance over which the electrostatic field of an ion exerts an appreciable effect. Figure 3 shows a theoretical plot of Debye length as a function of concentration of a univalent electrolyte in solution. The rapid decrease in Debye length between 0-20 mM electrolyte closely mirrors the change in viscosity seen in Figure 1 over the same electrolyte concentration range, and strongly suggests the viscosity effect seen in the present invention is due to electrostatic barrier effects.

F. Properties of the EGF/Gel Composition

Viscosity

The EGF/liposome gel composition of the invention is characterized by a high-viscosity gel-like consistency which is maintained at a low ionic strength, but which collapses as ionic strength is increased. This feature is illustrated in the study described in Example 2. Here liposomes containing equal-weight amounts of EPG, EPC, and cholesterol were prepared in a 2.3% w/v glycine buffer at isotonic pH (pH 6.0) buffer, as detailed in Example 1, except that EGF was not added. The mean viscosity for the samples was 13.3×10^3 Cps (centipoise) at 1.0 per second shear rate, characterized by a thick, relatively non-flowing gel consistency. With addition of NaCl to a concentration of only 0.05% w/v (at about 8.5 mM), the material lost its gel-like properties, being quite fluid, with a mean viscosity of only about 2.7×10^3 Cps at 1 per second. Further relatively small decreases in viscosity were seen with further addition of

NaCl to a final concentration of 0.2% w/v.

5 EGF Binding to Negatively Charged Liposomes

According to one aspect of the invention, it has been found that EGF may be entrapped in negatively charged liposomes by surface adsorption, and that the binding affinity of EGF for the liposomes is effective to produce slow release of adsorbed peptide both in vitro and in vivo. In the binding study reported in Example 4, liposome gel compositions formed from either PC/PG (equal weight ratios) or PC/PG/cholesterol (equal weight ratios) were prepared as in Example 1. Increasing amounts of EGF (iodine radiolabeled) were added to aliquots of each of the two compositions, and the mixtures were allowed to equilibrate for one week at 4°C. The ratio of bound to free EGF was determined from total radiolabel measured before and after centrifugation, and these values were plotted as a function of amount bound, yielding the plots in Figures 4 and 5 for the EPC/EPG and EPC/EPG/cholesterol compositions, respectively. Affinity constants K_d were determined from these plots as described in Example 4. As seen from the two figures, the K_d values are in the range $1-2 \times 10^{-5}$ molar for both compositions.

The number of EGF binding sites on the liposomes was determined from the x-axis intercept in the Figure 4 and 5 plots, along with the calculated K_d values, also as detailed in Example 4. From this, it was determined that at a peptide concentration of about 200 µg/ml, about 30% of the EGF is adsorbed at the lipid/water interface.

The adsorption of EGF to EPC/EPG and EPC/EPG/cholesterol monolayers was also examined in a lipid monolayer

system, also as detailed in Example 4. Briefly, the method measures the ability of EGF to interpenetrate the lipid monolayer, as evidenced by changes in the interfacial surface pressure as EGF is added to the monolayer.

5 Figure 7 is a plot of the interfacial surface pressure, π , as a function of lipid concentration for a EPC/EPG/cholesterol (equal wight ratios) lipid monolayers, as a function of lipid concentration. Similar plots were was made for EPC/EPG monolayers, and EPC/EPG/-
10 cholesterol and EPC/EPG monolayers containing 40 $\mu\text{g/ml}$ EPG, at each of several lipid concentrations. These plots were used to construct the graph of change in surface pressure due to the presence of 40 $\mu\text{g/ml}$ EGF in the monolayer, as a function of surface pressure, for
15 each of the two lipid compositions. This graph is shown in Figure 8.

Using linear regression analysis to extrapolate to the y-axis intercept it can be seen that the change in interface surface pressure produced by EGF in the EPC/-
20 EPG/cholesterol composition is about 15 dynes/cm, and in the EPC/EPG composition, about 13.5 dynes/cm. The interface surface pressure attributable to EGF alone (no lipid interaction) is plotted in Figure 6, and is $\mu.6$ dynes/cm at 40 $\mu\text{g/ml}$. Thus for both lipid compositions, the
25 measured change in surface pressure due to EGF in the presence of lipid is greater than that produced by EGF alone, indicating that the peptide is interacting with the monolayer.

The greater EGF-induced change in pressure seen in
30 the EPC/EPG/cholesterol composition indicates a greater degree of EGF interaction with the addition of cholesterol to EPC/EPG.

In vitro EGF Release Characteristics

The kinetics of release of EGF from EGF/liposome compositions prepared according to the invention were examined in a standard two-chamber percutaneous absorption cell, as detailed in Example 5. The samples placed in the donor cell were suspended in 25% human serum in isotonic saline, for passage across a membrane filter into a donor collector compartment which was continually perfused with 25% human plasma in saline.

Figure 9 shows release kinetics of EGF in the system for three independent kinetic studies. The mean half-life of EGF release, calculated from the slope of the availability of free EGF in the donor compartment, as a function of time, is about 1.8 hours.

The EGF available in the donor compartment from various EGF/liposome compositions were similarly measured. Figure 10-12 shows plots of EGF available in the donor compartment, as a function of time, from (Composition I) EPC/EPG liposomes with encapsulated EGF (Figure 10), (Compositions II) EPC/EPG/cholesterol liposomes with encapsulated EGF (Figure 11), and (Composition III) EPC/EPG/cholesterol with adsorbed EGF (Figure 12). All three compositions contain free EGF, and thus also are expected to contain liposome-adsorbed EGF.

The model used to determine the half-lives of EGF release from the liposomal formulations is discussed in Example 5. Briefly, the free EGF available in the donor compartment is determined from the measured rate of appearance of EGF in the receiver compartment, the rate constant K_p of the membrane, and the volume V_p of the external phase in the donor compartment. The calculated free available EGF in the donor compartment is then

plotted as a function of time, as seen in Figures 7-9. The half lives of EGF release during the slow phases is determined from the resulting plots.

The half lives determined from above are 14.1 hours for the EPC/EPG composition (encapsulated EGF); 10.1 hours for the EPC/EPG/cholesterol composition (encapsulated EGF); and 6.2 hours for the EPC/EPG/cholesterol composition (adsorbed EGF). It is clear that all of the liposome formulation enhanced the half life of EGF release in vitro severalfold over free EGF.

In vivo EGF Release Characteristics

The high-viscosity EGF/liposome compositions of the invention are effective to (a) remain physically localized at a site of injection or administration and (b) provide a source of therapeutic levels of EGF over a several-day period, as will now be demonstrated.

The enhanced retention of EGF in an EGF/liposome composition has been demonstrated with conjunctival placement of the various EGF compositions by sub-conjunctival-injection, and monitoring of levels of EGF retained at the conjunctival site over a several day period. The retention of radiolabeled EGF at a conjunctival site of administration, as a function of time after injection is shown in Figures 13-16 for free EGF (Figure 13), and EGF/liposomes composed of: (Composition I) EPC/EPG and containing free and encapsulated EGF (Figure 14), (Composition II) EPC/EGF/cholesterol and containing free and encapsulated EGF (Figure 15), and (Composition III) EPC/EPG/cholesterol and containing free and adsorbed EGF only (Figure 16). As seen, all of the EGF/liposome compositions give biphasic EGF release characteristics, indicating a burst of EGF released into the site of

administration, followed by a slow phase EGF release over a several-day period.

Table 3 in Example 6 gives the half-lives of EGF release, and the percent EGF released in the burst for free EGF and the three EGF/liposome compositions, calculated from the mean values of the data plotted in Figures 10-13. The half life of EGF retention was extended from 1 hour for free EGF to 14-35 hours for the liposomal compositions. Interestingly, and in contrast to the in vitro release kinetics observed, the largest half lives (32 and 35.6 hours) were obtained with Composition II and III (cholesterol-containing EPC/EPG liposomes), whereas the shortest half life (14 hours) was obtained with the Composition I. This discrepancy with the in vitro kinetics data may be due to the greater stability of cholesterol-containing liposomes in vivo, perhaps related to the reduced extent of lipid exchange which would be expected between liposomes and cells at the site of administration in the presence of cholesterol.

The long-term availability of EGF in the region of the EGF/liposomes is seen from the data in Table 4 of Example 6. For free EGF, substantially no EGF was available at the conjunctival site one day after administration. With the EGF/liposome formulations, more than 1% of the total EGF was available at the site 4 days after administration for Composition I, six days after administration, for Composition III, and seven days after administration, for Composition II.

From the foregoing, it can be appreciated that the degree of retention of EGF/liposomes at a site of administration can be selectively varied according to the amount of cholesterol included in the liposomes, at least over the range from about 0-33 weight percent.

The above results also indicate that liposome-adsorbed EGF in the EGF/liposomes is released from the liposomes in vivo at substantially the same rate as encapsulated EGF. This result confirms that EGF is tightly bound to negatively charged liposomes (containing at least 20 mole percent negatively charged phospholipid), and that an effective EGF/liposome formulation can be made by surface adsorption to liposomes.

10 G. Positively Charged Liposome Gel Composition

In another aspect, the invention includes a high-viscosity liposome gel composition for use either in applying lipid to a mucosal tissue, or in administering a liposome-entrapped drug to a mucosal surface tissue.

- 15 The composition includes a low-conductivity aqueous suspension medium having a selected pH between about 5.5 and 8.5, and between about 7-25 weight vesicle-forming lipids. The lipids contain between about 5-50 weight percent positively-charged vesicle-forming lipids, and
- 20 (ii) the balance of neutral vesicle-forming lipids. Preferably the positively charged lipids are the type described above which include a spacer at least 3 atoms in length greater between the lipophilic portion or moiety of the lipid and the positively charged polar head
- 25 group. These lipids provide enhanced liposome retention on mucosal surfaces. In particular, the positively charged lipid components in the composition preferably includes PE derivatives which are conjugated with basic amino acids, and/or amphophilic benzylyamine compounds,
- 30 as described above. Lipid compositions 9-12 above are exemplary.

The compositions are formed substantially as described in Section D, where the aqueous hydration medium

may either be a low-conductivity medium, or a medium containing a zwitterionic compound which allows titration to a low-conductivity medium. Example 7 illustrates a PC/BDSA gel composition formed by solvent injection.

5 In one embodiment, the gel composition is formulated as a lipid supplement for treatment of dry eye. Preferred lipid compositions are detailed in U.S. Patent No. 4,818,537 for "Method of Treating Dry". The present
10 differs from the earlier formulation in that high viscosity is produced by electrostatic effects rather than by high-viscosity polymers.

Alternatively, the positively charged liposome gel can be formulated to contain entrapped drug agents, for slow drug release from the liposomes.

15

II. Utility

A. Topical Administration

The liposome gel composition of the invention is useful as a moisturizing agent for application to dry or aging skin, and/or for applying cosmetic agents such as
20 vitamin A, UV-blocking agents, or retin, to the skin. The gel is easily delivered from a tube or the like, is relatively non-greasy to the touch, and is clear when applied to the skin. One unique property of the gel is
25 its ability to dissolve or melt over time as the gel becomes infused with salts on the skin. Thus, the viscous gel may be applied to the skin in gel form, but become quite fluidic as it is rubbed into the skin.

The gel may also be used as a drug delivery composition, for delivering a liposome-entrapped drug transdermally. The drug to be administered is typically a lipophilic drug, such as an anti-inflammatory steroid drug,
30 which is entrapped in the gel liposome lipids at a con-

centration between about 1-20 weight percent. It will be appreciated that a charged lipophilic drug may be administered, where the charge on the compound contributes to the liposome surface charge.

- 5 The EGF/liposome composition may be used in application to burns and other skin wounds, to promote healing. Epidermal Growth Factor (EGF), is a widely distributed endogenous polypeptide (King). It is a powerful mitogen with high affinity receptors in both fibroblasts and
- 10 epidermal keratinocytes, and has been shown to accelerate wound healing in vivo (O'Keefe; Knauer). The first 5-10 days after injury are the most critical period during which maximal differences are seen between EGF treated and untreated wounds. EGF application after this period
- 15 produces no significant improvement over controls, since by this time re-epithelialization has already occurred in both groups.

- For superficial wounds, local concentration of EGF can easily be maintained by applying the gel material
- 20 directly to the skin or in a skin dressing. The material is preferably supplied in gel form from a tube or the like which can be easily applied to the skin or to a skin dressing. One unique property of the gel material, when applied directly to the skin as a film, is that salts in
- 25 the skin will break down the gel structure, producing a fluid lipid dispersion as the material is rubbed in the skin..

B. Surgical Wound Administration

- 30 Because of its high viscosity, the gel composition is useful as a drug delivery vehicle for surgical wounds, where slow drug release over a several-hour to several-day period is required.

Due to its relatively short half-life of about one hour, (Buckley, 1987), loss of occupied receptors through turnover and a lag time of 8-12 hours to commit cells to DNA synthesis (Knauer), it has been necessary to apply EGF frequently to a wound to maintain effective local concentration during the critical period of initial wound healing (Buckley, 1987; Buckley, 1985; and Franklin, 1986). Thus, effective EGF therapy has required frequent or sustained application of the drug during the first several days of wound healing.

For surgical incisions and full thickness skin wounds requiring suture repair, frequent application of EGF is not possible and a sustained-release formulation of EGF must be used for these uses. Implanted sponges have demonstrated the advantages of sustained EGF release in an animal wound model (Buckley, 1985) but would not be suitable as a dosage form.

The EGF/gel composition of the invention substantially overcomes these limitations. In practice, the gel composition is applied incision area before suturing. The high viscosity of the of the material reduces loss of material from the incision site, and the slow release of EGF from the liposomes provides a therapeutic level of EGF at the site over a several-day healing period.

C. Ophthalmic Uses

The gel composition of the invention also provides a number of advantages for lipid or drug administration to mucosal tissue. For treatment of dry eye, where the gel serves as a source of lipid and moisture, the gel has the advantages of optical clarity, and enhanced retention due to high viscosity. Further, where the liposomes contain

positively charge lipid elements with charge spacers, as described above, the liposomes themselves have enhanced retention to corneal surfaces. The liposomes may also be used for drug delivery to the eye or other mucosal surface, with advantages of greater retention, i.e., less flow from, the site of application.

Figures 17A-17C illustrate surgical incision and incision repair step in a corneal replacement or transplant operation. An initial arcuate incision in the conjunctiva, illustrated in Figure 17A, allows the conjunctiva to be pulled away, exposing the underlying episclera and cornea. A second arcuate cut in the cornea, shown in Figure 17B, allows the cornea to be pulled back to provide access to the lens (not shown). After surgical removal or replacement of the lens, the cornea is first closed by stitching, seen at 20 in Figure 17C, followed by closure of the conjunctiva by stitching, indicated at 20. Post-operative healing involves healing of the two incisions, and regrowth of the episclera layer between the conjunctiva and cornea.

Figures 18A-18C illustrate the use of the EGF/liposome composition of the invention to promote healing of the above-described ophthalmic surgery. Figure 18A shows a cross section through an outer portion of the stitched cornea and conjunctiva, as seen in perspective in Figure 17C. After stitching, the space between the conjunctiva and cornea is filled with an EGF/liposome composition, by inserting a needle through a region of the stitched incision in and injecting the composition into the episclera space. As illustrated in Figure 18B, the material may be injected until a slight bulging of the conjunctiva is produced.

The injected material remains in place, over a several-day period, by virtue of its gel or paste-like consistency. EGF is released into the surrounding area, promoting healing of both stitched incisions and regrowth of the episclera over an extended healing period.

Preliminary studies conducted in support of the present invention have examined the effect of viscous EGF/liposomes on ophthalmic incision repair in an animal model system. Briefly, it has been found that a viscous EGF/liposome composition provides greater wound repair, as measured by the strength of the repaired incision several days after treatment, than empty liposomes.

According to another important advantage, the gel composition of the invention combines high viscosity with low lipid concentration, so that the material is relatively inexpensive in terms of materials cost. Further, additives, such as high molecular weight polymers, colloids and the like, are avoided.

The following examples are intended to illustrate various compositions, methods of preparations, and characteristics of the present invention. The examples are in no way intended to limit the scope of the invention.

Example 1

Preparation of EGF/Liposome Compositions

EPG was purchased from Avanti Polar Lipids (Birmingham, AL) and EPC was purchased from Asahi Chemical Company (Tokyo, Japan). Cholesterol was from Croda, Inc. (New York, NY) and α -tocopherol (Vitamin E) from Hoffman - La Roche (Nutley, NJ). Aminoacetic acid (glycine) was from J.T. Baker (Philipsburg, NJ).

EGF from yeast (Chiron) was a gift of Ethicon, Inc. (Somerville, NJ). ^{125}I -labeled rh-EGF (3-[^{125}I] iodotyrosyl

human recombinant epidermal growth factor was purchased from Amershal Corporation (Arlington Heights, IL). ¹²⁵I rh-EGF was shipped the day of iodination and used only for the first four weeks following iodination.

5 Liposomes were prepared by thin film hydration of a dehydrated lipid mixture containing one of the following lipid mixtures. Composition I: EPG/EPC/ α -tocopherol (1/1/0.03, w/w/w) and Composition II: EPG/EPC/cholesterol/-tocopherol (1/1/1/0.03, w/w/w/w). The lipids
10 were dissolved in chloroform:methanol (2:1) and a total of 33 g of lipid were added to a round bottom flask and dried in vacuo to a thin film. To this film was added 267 ml of hydration buffer containing 2.3% (w/v) glycine, pH 6.0. Hydration was carried out for 1-2 hours with
15 swirling. The material had a stiff, gel-like consistency.

The materials were prepared in a biological cabinet using sterilized equipment, filter-sterilized lipid, and filter-sterilized aqueous solutions to keep the bioburden
20 as low as possible. The vesicles were prepared by thin-film hydration in a 2.3% glycine buffer. The resulting liposome dispersion was injected by extrusion through a Gelman Acrodisc into 1 or 10 ml plastipak syringes which were wrapped in aluminum foil and labeled. An aliquot of
25 the liposome gel was set aside, and "collapsed" back into a lotion by the addition of concentrated saline.

Samples were assayed for rh-EGF concentration, total lipid phosphate, cholesterol content, pH, viscosity, osmolality, particle size and pyrogen levels (Table 2).
30 Mean diameters were assayed using the Nicomp laser particle sizer.

TABLE 2
Characterization of EGF Compositions

	Assay (units)	<u>rh-EGF-Loaded</u>		<u>Placebo</u>	
		<u>"Lotion"</u>	<u>"Gel"</u>	<u>"Lotion"</u>	<u>"Gel"</u>
5	rh-EGF ($\mu\text{g/gm}$)	192	197	0	0
10	Total Lipid Phosphate ($\mu\text{mol/gm}$)	91.1	100.4	87.0	87.0
	Cholesterol (mg/gm)	31.6	31.6	34.3	32.2
15	Buffer pH	6.1	6.1	6.0	6.0
	Osmolarity (mOsm)	326	313	319	305
20	Viscosity (Cps) ^(c)	2,750	17,700	3,600	20,500
25	Nicomp Mean Diameter (nm)	630	713	644	666
30	LAL Pyrogen Test	Pass	Pass	Pass	Pass

Example 2

Viscosity of the EPG Gel Liposome Composition

35 Five separate batches composed of EPG/EPC/ cholesterol/ α -tocopherol (1/1/1/0.03, w/w/w/w) liposomes were prepared as described in Example 1. The viscosity of each of the batches was determined (a) without addition of NaCl, and after addition of (b) 0.05%, (c) 0.1, and
40 (d) 0.2% by weight NaCl. At each salt concentration, the mean viscosity of the compositions tested was determined. The measured values, expressed as extrapolated Cps at 1

per second shear rate, are shown in Figure 4 and in Table 3 below.

Viscosity was determined using a Brookfield DV-II cone/plate viscometer. Viscosity readings were made at all relevant spindle speeds. Spindle speeds were converted to the shear rate. Plots of log (viscosity) versus log (shear rate) were prepared from which the viscosity at a shear rate of one reciprocal second was extrapolated.

Table 3

	<u>% NaCl</u>	<u>Mean Viscosity (Extrapolated Cps at 1 sec. shear rate)</u>
15	0.0 %	13.3×10^3
	0.05%	2.7×10^3
20	0.1 %	1.5×10^3
	0.2 %	0.8×10^3

The mean viscosity of the composition in the absence of NaCl corresponds to a stiff, gel-like consistency. As seen, addition of only a slight amount of salt reduces the viscosity severalfold, producing a thinner, lotion-like consistency.

Example 3

Processing EPG Liposome Gel Composition

Liposomes were prepared by thin film hydration of a dehydrated lipid mixture containing EPG/EPC/cholesterol/ -tocopherol (1/1/1/0.03, w/w/w/w), as described in Example I, except that the hydration buffer used to

produce the liposomes contained 50 mM glycine, adjusted to pH 8. The liposome suspension was highly fluidic.

The suspension was sized by extrusion by multiple through a 0.2 micron polycarbonate membrane. The sized liposomes were then sterilized by filtration through a 0.25 micron depth filter. To this sterilized material was added 1/10 volume of sterilized 10X glycine, adjusted to produce a final pH of 6.0. The final suspension had a stiff, gel-like consistency.

10

Example 4

Adsorption of EGF to Liposomes

A. Scatchard Analysis

The affinity of EGF binding to liposomes and the number of binding sites in the Example 1 liposomes can be determined from Scatchard analysis of the binding of radiolabeled rh-EGF to the liposomes.

Liposomes formulations I and II in Example 1 were prepared by hydration of the thin lipid film with 2.3% glycine (w/w), as described in Example 1. The formulations were sized (three passes for each pore size) sequentially through 5.0 μm and then 1.2 μm polycarbonate filters, then extruded through a 0.4 μm Nuclepore filter.

Five μCi of ^{125}I -rh-EGF were added to 2 ml of rh-EGF, 1 mg/ml concentration in glycine buffer. Aliquots of this iodinated stock solution were added to duplicate, 1 ml samples of the liposome preparation also containing five final rh-EGF concentrations ranging from 6 to 100 μg rh-EGF per ml liposomes. The resulting preparations were allowed to come to equilibrium by incubation for one week at 4° C.

30

After equilibration, known volumes of the preparations were removed for gamma counting. A known volume of the bulk of the remainder of each sample was centrifuged for two hours at 40K RPM and 4° C. Known volumes of the clear supernatant ("free" EGF) were removed for gamma counting. Any remaining supernatant was removed and discarded. Each liposome pellet was resuspended in 1.2 ml of 1% (w/w) Triton X-100 and vortexed. All samples were then assayed for gamma counts, and the actual CPM per ml were determined.

The ratio of bound/free was determined for each sample as:
$$\text{Ratio} = [\text{bound}] / ([\text{bound plus free}] - [\text{bound}]) \quad (\text{Eqn. 2})$$

Values of [bound]/[free] versus [bound] were plotted and the data fit to a linear least squares regression. Figures 4 and 5 show the plots for the I and II formulations, respectively. K_d , the binding constant of the peptide on the liposomes was determined from the slope of the regression line, which was taken to be $1/K_d$. Confidence intervals of K_d were calculated according to known methods (Tallarida).

The results seen in Figures 4 and 5 indicate that there is no significant difference between the two formulations as to affinity constants, which is calculated as about $1-2 \times 10^{-5} \text{ M}$ for both formulations. The K_d for liposomes is several orders of magnitude less than that for cultured fibroblasts ($2 - 4 \times 10^{-10} \text{ M}$) (Buckley, 1987).

The number of binding sites was determined from the X-axis intercept of the of the regression line, which was taken to be equal to [binding sites] K_d (Scatchard). The EPC/EPG formulation had 0.8 μg EFG binding sites per mg

lipid, and the EPC/EPG/cholesterol formulation had 1.4 μg EGF binding sites per mg lipid. The number of binding sites is actually the number of binding sites on the external face of the lipid bilayer. Thus, for large
5 unilamellar vesicles, the actual number of binding sites would be twofold greater than for multilamellar or oligolamellar preparations like these, at least threefold greater. The lipid concentration used is sufficient to potentially adsorb all the rh-EGF.

10 Knowing the estimates for K_d and the number of binding sites, it can be calculated that at this lipid concentration and at a peptide concentration of about 200 μg rh-EGF/gm formulation, about 30% of total rh-EGF is adsorbed at the lipid/water interface.

15

B. Surface Pressure Measurements

Adsorption of native rh-EGF lipid monolayers to lipid/water interfaces can be evaluated by measuring surface tension of lipid monolayers spread on an rh-EGF-
20 containing aqueous subphase. The methodology of Weiner and coworkers (Schwinke) was used to rank order different lipid monolayer compositions with respect to the enhanced ability of a peptide to interpenetrate a given monolayer. Distilled water adjusted to pH 6.0 was used as the sub-
25 phase in these pilot experiments. Other experiments done in 2.3% glycine as buffer gave the same results.

Surface tension measurements were made on a CSC Scientific Model 70545 DuNouy tensiometer (Fairfax, VA). Briefly, a new lipid monolayer was spread from a hexane/-
30 ethanol (95/5, v/v) solution for each determination. Surface pressure (π) was determined as the difference of the surface tension of test monolayer of subphase minus

the surface tension of subphase alone. $\Delta \pi$ is the difference between mean monolayer surface pressure in the presence and absence of rh-EGF. All data points are the mean of at least duplicate determinations.

5 Figure 6 is a plot of surface pressure π of aqueous EGF measured as a function of EGF concentration. As illustrated in the figure, rh-EGF is a surface active peptide having a limiting pressure of 12.6 dynes/cm as determined from a double reciprocal transformation of
10 this graph. Surface pressure changes also be monitored as a function of lipid concentration. As an example, EPG/EPC/Chol (1/1/1, w/w/w) monolayers displayed a limiting pressure of 50.9 dynes/cm in the absence of rh-EGF in the aqueous subphase (Figure 7). This limiting pressure
15 (also called the collapse pressure) is considered to be the equilibrium pressure of liposome bilayers (MacDonald).

 The ability of the peptide to interpenetrate the lipid layer can be determined, as indicated above, by
20 measuring interfacial surface pressure in the presence and absence of peptide at several lipid concentrations. The difference in surface pressures ($\Delta \pi$) is plotted versus the surface pressure in the absence of peptide.

 In Figure 8, data for EPG/EPC (1/1, w/w) and EPG/-
25 EPC/Chol/a-toc (1/1/1, w/w/w) are plotted. The rh-EGF concentration in the subphase was 40 ug/ml. A linear regression analysis was carried out on the data to extrapolate to the y-axis intercept (limiting π) (Wiener). The fact that this value, e.g., 15 dynes/cm for the
30 EPC/EPG/cholesterol formulation, is greater than the peptide's own limiting pressure in the absence of lipid monolayer, is evidence that the peptide is penetrating

the lipid monolayer. That is, the observed effect is not merely competition for the surface by two different surface-active molecules (Schwinke). Furthermore, the formulations may be rank-ordered according to the magnitude of the extrapolated peptide/lipid limiting π .

Based on this analysis, both Composition I (solid circles in Figure 8) and Composition II (open triangles in Figure 8) show rh-EGF adsorption to the lipid. The greater extrapolated value of $\Delta \pi$ for the EPC/EPG/-cholesterol formulation suggests that this composition formulation shows a greater degree of peptide/bilayer interaction than the EPG/EPC (1/1, w/w) formulation.

Example 5

In vitro EGF Release Kinetics from EGF/Liposomes

Liposome formulations were evaluated in vitro by release rates of 125 I-rh-EGF into the receiver compartments of percutaneous absorption cells that were continuously perfused with 25% pooled human plasma/saline.

A conventional two-chamber absorption cell, using a bath temperature of 37°C, was employed (Bronaugh). A 25 mm, 0.08 μ m diameter Nuclepore (Pleasanton, CA) polycarbonate filter was used to separate the donor from the flow-through acceptor compartment. This pore size gave the shortest half life of free EGF passage --about 1.8 hours-- without detectable passage of intact liposomes through the membrane. The ¹²⁵I-rh-EGF preparation was mixed with an equal quantity of 25% pooled human plasma/isotonic saline and 200 μ l aliquots were immediately applied to the donor compartments. Parafilm was used to occlude the donor compartment and prevent evaporative loss. The perfusate was collected by fraction collector

into scintillation vials and counted in a Packard TriCarg 20000. rh-EGF flux was calculated from the specific activity (DPM per μg peptide). Clearance rate half-lives were determined for individual percutaneous absorption

5 cells as described below.

Figure 9 shows the available radiolabeled rh-EGF in the donor compartment of three Bronough cells, as a function of time, for an rh-EGF solution. In this single phase system, it is possible to determine the concentration of peptide remaining in the donor compartment by subtracting cumulative peptide in the measured in the receiver compartment from total peptide applied to the donor compartment. The half-life of EGF release determined from the mean of the three EGF curves, is about 1.8
10 hours. Since the membrane is rate-limiting for EGF transfer from the donor to receiver compartment, the curve can also be used to calculate K_b , the rate constant of the membrane.

The determination of drug-release halflife from the
20 pool of free EGF available in the donor compartment is more complicated, due to the fact that in EGF/liposomes, only EGF which is located in the external aqueous phase is potentially bioavailable. A model proposed by Chowhan et al for solute flux from a liposomal carrier is represented by the following equation:
25

$$C_{II} = (dAs/dt) / (K_b * V_b) \quad (\text{Eqn.1})$$

where, C_{II} is the concentration of the solute of interest in the external aqueous phase, dAs/dt is the rate of solute appearance in the receiver compartment during the
30 time period of interest, K_b is the rate constant of the membrane separating donor and receiver compartments, and V_b is the volume of the external phase in the donor compartment. The rate of solute appearance in the donor

compartment was determined, as above, by assaying the amount of radiolabel in the donor compartment over the efflux period. K_d was determined from the free drug efflux study above, and V_d can be estimated to within 5 about 10%. Using the equation above, the C_{II} , the available free EGF in the donor compartment, was calculated as a function of time. Figure 10 shows plots for seven Brnough cells, where the heavier line represents the overlap of two or more plots.

10 The release profile of all liposome formulations was best modeled by bi-exponential fit. The slow-phase $t_{1/2}$ of Composition I EPG/EPC/a-toc (1/1/0.03, w/w/w) MLVs with free and entrapped rh-EGF was about 14.1 hours. "Free" rh-EGF in the external phase of the donor compartment was maintained above a concentration of 0.063 μ g 15 EGF/ml (the lower threshold of therapeutic activity) for over 50 hours.

Figure 12 shows similar plots from five Brnough cells of the availability of free EGF in the donor cell, 20 as a function of time, for an EGF/liposome composition having the Composition II formulation EPG/EPC/Chol/a-toc (1/1/1/0.03, w/w/w/w). As with the composition above, the liposomes contained liposome-encapsulated EGF, as well as liposome-adsorbed and free EGF.

25 The available, i.e., free EGF, in the donor compartment was determined as above. From the plots, a mean half-life of about 10.1 hours for the slow phase of the release kinetics was observed. Thus, free rh-EGF displayed a somewhat shorter $t_{1/2}$ than for the liposome formulation lacking cholesterol, although the two half-lives 30 are not statistically different.

In another study, rh-EGF was added to pre-formed EPG/EPC/Chol/a-toc (1/1/1/0.03, w/w/w/w) MLVs to give an

initial external phase peptide concentration in the vicinity of 100 ug/gm formulation. The slow phase $t_{1/2}$ of this formulation (Composition III) was significantly longer (6.2 hrs, Figure 12) than that of the free rh-EGF solution tested in the same model, and somewhat less than the above liposome compositions containing both encapsulated and liposome-entrapped EGF. The data indicate that release of adsorbed EGF from liposomes is rate limiting and occurs with roughly the same release kinetics as EGF which is both encapsulated in and adsorbed to the liposomes.

Example 6

In vivo EGF Release Kinetics from EGF/Liposomes

The EGF/liposome compositions tested are given in Section 1B above. All liposome formulations consisted of unsized multilamellar vesicles containing egg phosphatidyl glycerol and partially hydrogenated egg phosphatidyl choline, prepared substantially as in Example 1. All formulations contained glycine buffer (2.3%) as a gelling agent and α -tocopherol (0.1%) as an antioxidant. All formulations contained ^{125}I -h-EGF (0.01 uCi/ug) as a radiolabel, and some contained a small amount of tritiated cholesterol (<0.1% of total lipid) as a formulation marker. Free EGF and Composition I contained Thimerosal (0.1%) and dimethylene-triaminetetraacetic acid (DTPA) (0.1%), but Compositions II and III did not. In addition Compositions II and III were prepared aseptically in an attempt to minimize irritation following administration. Compositions II and III had the same lipid composition but were loaded differently. EGF was encapsulated into Composition II liposomes during formation, and therefore contained entrapped drug. EGF was added to the

liposomes of Composition III after formation, and was therefore considered adsorbed to the liposome surface. Total EGF was adjusted to give similar quantities of EGF in the external aqueous phase in both compositions II and III.

Table 4

CHARACTERISTICS OF EGF FORMULATIONS

FORMULATION				
	Free EGF	Liposome I	Liposome II	Liposome III
Type	Free	Adsorbed and Entrapped	Adsorbed and Entrapped	Adsorbed
EPC, mg/g	0	130	35	35
EPG, mg/g	0	130	35	35
Cholesterol mg/g	0	0	35	35
EGF, ug/g	100	100	100	40
DTPA + Thimerosal	Yes	Yes	No	No
Aseptic Process	No	No	Yes	Yes
pH	7.0	6.05	6.02	6.02
EGF Dose, ug/kg body wt.	2.3	2.1	1.6	0.63

Formulations of free or liposome associated ^{125}I EGF were administered by subconjunctival injection to female New Zealand White Rabbits (2-3 kg). Conscious rabbits were placed in a bag restrainer, and 1-2 drops of Ophthalmine 0.5% Ophthalmic Solution were administered to the eye as a local anesthetic 1 minute prior to the injection.

tion. A small fold of conjunctiva superior to the cornea was raised with forceps and 50 ul of the formulation injected slowly through a 27 ga needle using a calibrated glass microsyringe (Hamilton Co., Reno, NV). Both left
5 and right eyes were used on separate days in some rabbits but no eye was used more than once during the study. Eyes were monitored for signs of leakage, inflammation or irritation. Small blebs formed following injection which tended to migrate slowly downward over a period of days.
10 A liposome encapsulated dye appeared to remain localized in the eye for over 5 days following injection in one rabbit, thus indicating the ability of a formulation to remain physically localized at the injection site during the study.

15 The amount of radioactivity remaining in the injected eye was measured for up to 96 hours after injection (until at least 90% of the initial radioactivity had disappeared). An external NaI crystal detector/ratemeter (The Nucleus Model L) was placed directly over the eye
20 and radioactivity measured for 2 minutes and recorded on a chart recorder. A standard curve was prepared by serial dilutions of the dose solution to calibrate and demonstrate the linearity of the detector response. The mean chart deflection during each recording was taken as
25 the measurement at that time point. The first reading was taken immediately after injection ("zero time") and subsequent measurements were converted to a percentage of this initial amount.

Individual radioactivity versus time data were
30 recorded for each animal, and the mean values for each animal group (N= 4-6) were calculated. These data are plotted in Figures 13-16 which show the remaining ¹²⁵I-rh-EGF in conjunctiva as a function of time following sub-

conjunctival injection of (a) free EGF (Figure 13), (b) Composition I EGF/liposomes (Figure 14), (c) Composition II EGF/liposomes (Figure 15), and (c) Composition III EGF/liposomes (Figure 16). As seen from Figure 11, un-encapsulated EGF disappeared rapidly from rabbit eyes after subconjunctival injection (Fig.1). This disappearance was first-order and had a half life of 1.0 hours. Less than 15% of the dose remained in the eye after 3 hours, with only $1 \times 10^{-5}\%$ expected to remain after 24 hours. In contrast, EGF administered in liposome formulations disappeared much more slowly from the eye. Formulation I (EPC/EPG) exhibited a small initial burst disappearance followed by first-order disappearance with a half-life of 14.1 hrs (Figure 11). Formulations II and III (EPC/EPG/CH) both exhibited a larger initial burst followed by a slower first-order disappearance (Formulation II half-life: 32 hrs, Formulation III half-life: 35.6 hrs).

Each plot was used to calculate first order rate constants for EGF disappearance from the eye for each formulation by a non-linear least squares fitting method (RSTRIP, MicroMath, Salt Lake City, UT). Where a significant initial burst release was observed, only the log-linear portion of the curve after the rapid initial phase was fitted. The calculated rate constants, half-life, and % of dose in the burst release are given in Table 5 below.

Table 5
IN VIVO PERFORMANCE OF LIPOSOME EGF FORMULATIONS

	Free EGF	FORMULATION		
		I (No cholesterol)	II (entrapped)	III (adsorbed)
5				
10	Rate constant hr ⁻¹ (a)	0.69	0.049	0.022
	Half-life, hrs	1.0	14.1	32.0
	Burst Release % of Dose	N/A	15.0	43.0
				57.0

(a) Estimated from non-linear least squares fit of log-linear portion of disappearance curve following initial burst (see text).

Since the half-life of disappearance of free EGF was 1.0 hr, it is reasonable to assume that the release of EGF from the liposomes is the rate limiting step in the prolonged disappearance of EGF from the eye observed in liposome-containing formulations, and that observed disappearance half-lives approximate half-lives of EGF release from the liposomes. From the first order rate equation:

$$A_t = A_0 * e^{-kt} \quad \text{or} \quad \ln(A_t) = \ln(A_0) - kt$$

where $k = 0.693 / t_{1/2}$, the amount of EGF lost from the eye during any day can be calculated. For example, the amount of EGF lost on day two (24-48 hrs) for Formulation I ($k = 0.049 \text{ hr}^{-1}$, $A_0 = 85\%$) is founded by taking the difference between A_{24} and A_{48} where $A_{24} = 85 * e^{-0.049*24} = 26.2$ and $A_{48} = 85 * e^{-0.049*48} = 8.09$. This difference is 18.1% of the total dose administered.

Using the first-order rate constants from Table 5, and estimating A_0 as the total dose minus burst, the

percentage of the administered EGF dose lost on each day was estimated (Table 6).

Table 6

5	FORMULATION				
	FREE EGF	I (No cholesterol)	II (entrapped)	III (adsorbed)	
10	%Lost: (a)				
	Day 1	100	73.8	63.6	71.8
	Day 2	0	18.1	14.3	10.5
15	Day 3	0	5.6	8.7	6.6
	Day 4	0	1.7	5.3	4.1
20	Day 5	0	0.53	3.2	2.6
	Day 6	0	0.16	1.9	1.6
25	Day 7	0	0.05	1.2	1.0

(a) Based on first order rate constants shown in Table 5 (see test for details of calculations). Day 1 values include initial burst. Values are % of total dose administered.

All three EGF/liposome compositions gave at least 1% per day for the first four days, and Composition II and III continued to deliver this amount of EGF for up to 7 days.

The addition of cholesterol reduced the rate of EGF disappearance in liposomes, but increased the initial burst release (up to 57% in the first 40 minutes). This burst effect may be due to release of adsorbed drug from the outer bilayer(s) during initial in vivo destabilization, but it is not clear why burst release was lower

with the cholesterol-free formulation (I). The effect of a burst release is to rapidly elevate local drug concentration after injection. Therefore, a certain degree of burst release may be advantageous in establishing rapid onset of action. Even though they lost more of their contents early, the cholesterol-containing formulations (II & III) were able to deliver more drug on days 3 to 7 due to their slower release rate.

Local erythema and edema were observed in some rabbits following administration. All of the rabbits receiving free EGF displayed redness at 90 minutes and increasing edema by the end of the study (3 hours), at which time they were treated with ophthalmic ointment. Following injection of Formulation I, all eyes appeared redened at 90 minutes, erythema and edema increasing to a maximum at 6 hours and then subsiding. Free EGF and Composition I contained Thimerosal and DTPA, and were not prepared aseptically. Composition II and III were subsequently prepared aseptically without Thimerosal or DTPA. These formulations caused mild erythema in 6 of 8 rabbits, but edema was observed in only one animal, indicating reduced irritation compared with the first two formulations.

Both Compositions II and III appeared to release EGF at approximately the same rate despite the fact that Composition II contained entrapped and absorbed drug while Composition III contained only adsorbed drug. This may indicate that EGF (an amphipathic peptide) is strongly adsorbed to the bilayer, as discussed in Example 3 above, and that the rate limiting step in its release involves desorption from its membrane associated state, rather than "leakage" through the lipid barrier.

Example 7Positively Charged Liposome Gel Composition

Fully hydrogenated soy PC (HSPC) were obtained from American Lecithin Company (Atlanta, GA). Benzyldimethyl-
5 stearylammonium chloride (BDSA) was obtained from Aldrich Chemical Company (milwaukee, WI).

A lipid mixture containing 32 g HSPC and 4 g BDSA was dissolved in 38 ml ethanol, at about 60° C. This lipid solution was then injected slowly, with stirring,
10 into 500 ml of distilled water, also at 60° C. The liposome suspension which was produced was cooled to room temperature, resulting in a substantially non-flowing gel.

Although the invention has been described with re-
15 spect to particular embodiments, methods, and applications, it will be apparent that various changes and modifications can be made without departing from the invention.

IT IS CLAIMED:

1. A high-viscosity liposome gel composition comprising
 - 5 (a) a low-conductivity aqueous suspension medium having a selected pH between about 5.5 and 8.5, and
 - (b) suspended in the medium, at a concentration of between about 7 to 25 weight percent total lipid, liposomes composed of (i) between about 5-50 weight percent
10 charged vesicle-forming lipids which contribute a common net charge to the outer surfaces of the liposomes, at the selected pH, and (ii) the balance of neutral vesicle-forming lipids.
- 15 2. The gel composition of claim 1, wherein the aqueous medium contains a zwitterionic compound whose isoelectric point is substantially at the selected pH.
- 20 3. The gel composition of claim 2, wherein the zwitterionic compound is a neutral amino acid.
4. The composition of claim 3, wherein the concentration of zwitterionic compound is such as to produce a substantially isotonic medium.
- 25 5. The composition of claim 1, wherein the charged vesicle-forming lipids phosphatidylglycerol, and the neutral vesicle-forming lipids include phosphatidylcho-
line.
- 30 6. The composition of claim 5, which further includes liposome-entrapped epidermal growth factor, and

wherein the concentration of phosphatidylglycerol in the liposomes is between about 10-50 weight percent.

7. The composition of claim 5, wherein the liposomes are composed of between 20-40 weight percent each of phosphatidylglycerol, phosphatidylcholine, and cholesterol.

8. The composition of claim 1, for use in applying liposomes to a mucosal tissue, wherein the charged vesicle-forming lipids are positively-charged components.

9. The composition of claim 8, wherein the charged lipids include a phosphatidylethanolamine conjugate prepared by derivatizing phosphatidylethanolamine with a basic amino acid.

10. The composition of claim 8, wherein the charged lipids includes a benzylamine lipid.

11. An epidermal growth factor (EGF)/liposome composition having a gel-like consistency, comprising

(a) liposomes containing between 20-50 mole percent each of phosphatidylcholine, phosphatidylglycerol, and cholesterol, at a total lipid concentration of between about 50-200 mg/g composition;

(b) a low-conductivity aqueous medium containing a zwitterionic compound whose isoelectric point is between about pH 6-8; and

(c) liposome-entrapped EGF, at a concentration between about 0.5 - 5 µg/ml.

12. A method of preparing a liposome gel composition, comprising

mixing a lipid composition containing between about 5-50 weight percent charged vesicle-forming lipids having a common charge at a selected pH between about 5.5 and 8.5, and the balance of a neutral vesicle-forming lipids with a low-conductivity aqueous suspension medium, at a total lipid concentration of between about 7-25 weight percent.

13. The method of claim 12, wherein the aqueous medium contains a zwitterionic compound whose isoelectric point is between about pH 5.5 and 8.5.

14. The method of claim 13, wherein said mixing includes adding the lipid composition to an aqueous medium whose pH is substantially different from the isoelectric point of the zwitterionic compound, thereby to form a substantially fluidic liposome suspension, and adjusting the pH of the suspension to the isoelectric point of the zwitterionic compound, to produce the desired gel-like suspension.

15. The method of claim 14, which further includes processing the relatively fluidic liposome suspension to achieve a desired liposome size change prior to said adjusting.

16. A method of forming an epidermal growth factor (EGF)/liposome composition having a gel-like consistency, comprising

(a) providing a lipid mixture containing neutral phospholipid, between 10-50 weight percent negatively charged phospholipid and EGF; and

- (b) suspending (i) the lipid mixture to a final lipid concentration between about 50-200 mg/ml, and (ii) EGF, to a final EGF concentration of between about 0.1 and 10 µg/ml, in a low-conductivity aqueous medium containing a zwitterionic compound having an isoelectric point between pH 5.5-8.5.

10

17. The method of claim 16, wherein said peptide is added after formation of liposomes in the aqueous medium, and the polypeptide is predominantly adsorbed to the liposome surfaces.

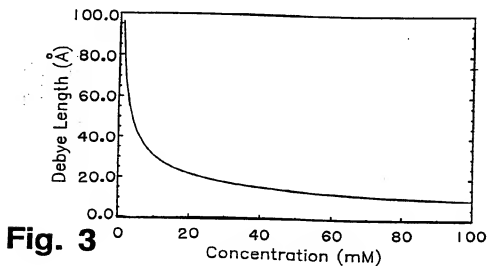
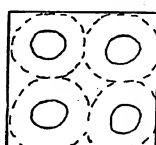
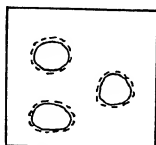
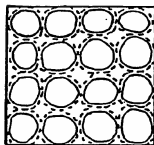
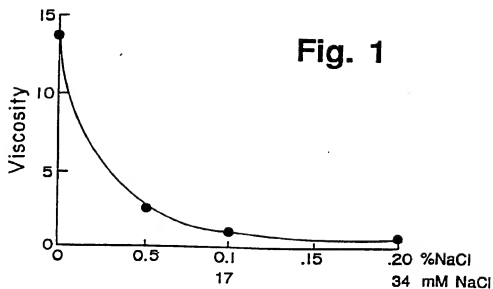
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18. A method of treating a wound or surgical incision with a sustained release dose of epidermal growth factor (EGF),

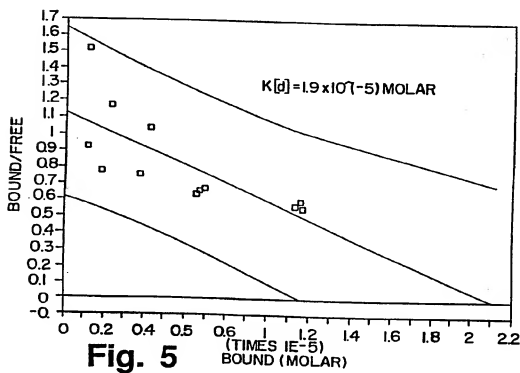
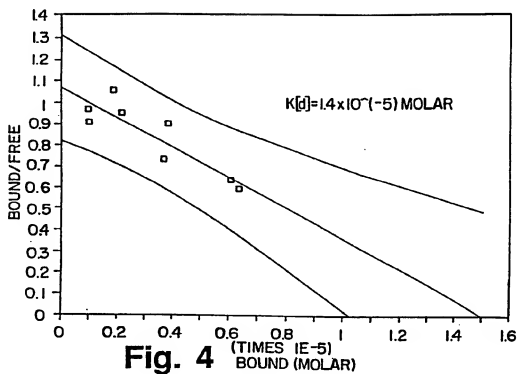
- providing a high-viscosity liposome composition comprising (a) EGF/liposomes (i) containing neutral phospholipid, at least about 10 weight percent negatively charged phospholipid, and liposome-entrapped EGF, and (ii) having a lipid concentration of less than about 200 mg/g composition and a pH between 5.5 and 8.5 and (b) means for imparting a high composition viscosity selected from the group consisting of (i) an low-conductivity aqueous medium containing a zwitterionic compound having an isoelectric point between 5.5 and 8.5; and (ii) empty liposomes which are substantially free of negatively charged phospholipid and liposome entrapped EGF, and applying the composition to the wound or incision site.

19. The method of claim 18, for treatment of surgical incision, wherein the composition is applied between surgical incision layers.

- 5 20. The method of claim 19, for treatment of ophthalmic surgical incisions involving both corneal and conjunctiva incisions, wherein the composition is injected to the space between the cornea and conjunctiva, after resuturing the conjunctiva.

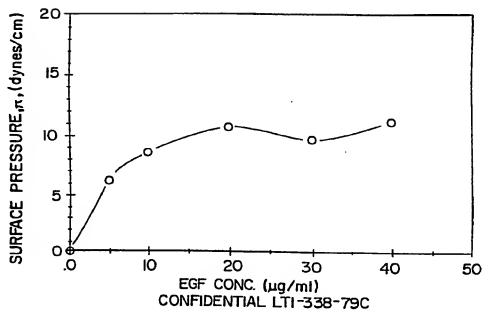
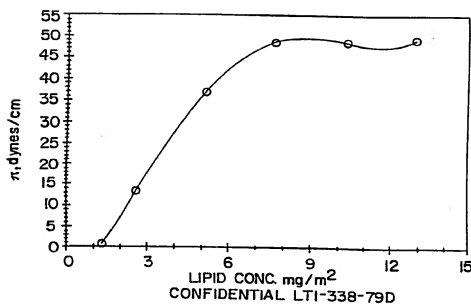


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**Fig. 6****Fig. 7**

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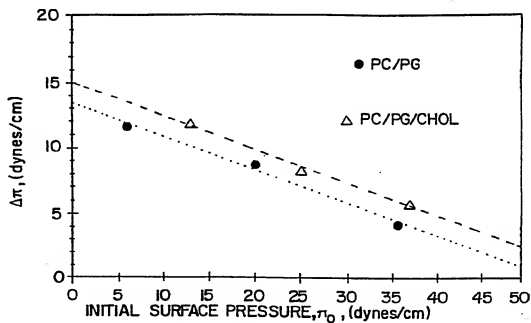


Fig. 8

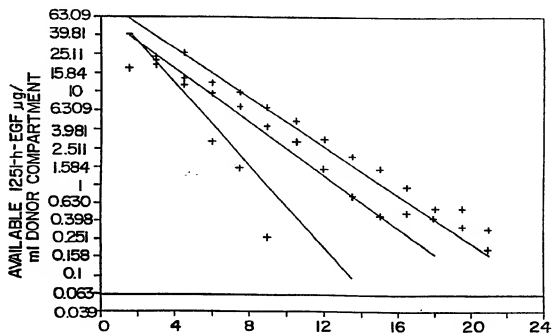


Fig. 9

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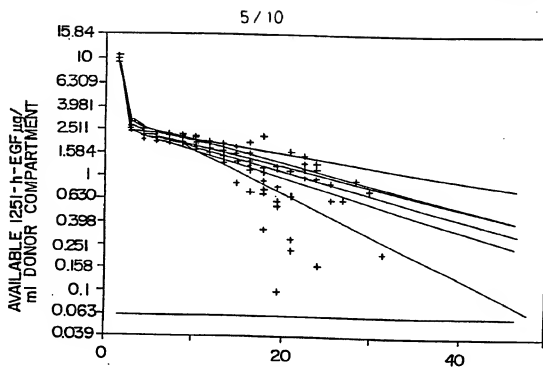


Fig. 10

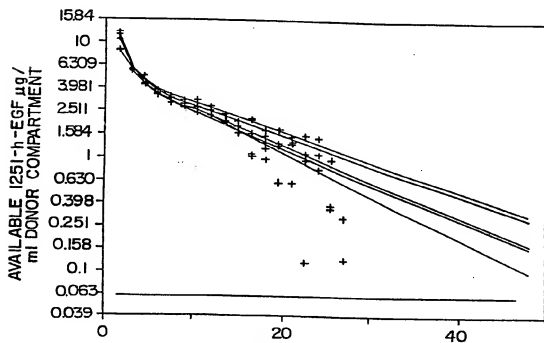
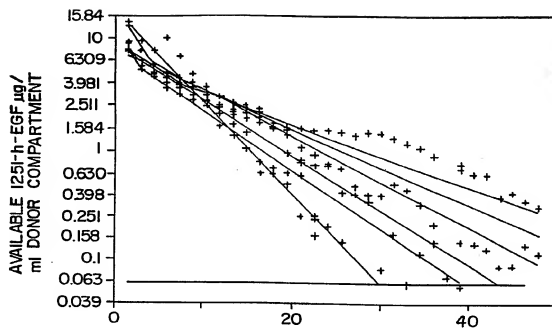
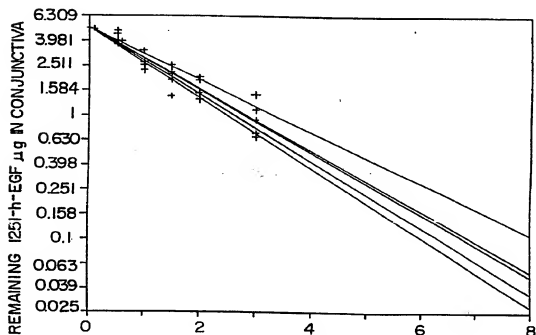


Fig. 11

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**Fig. 12****Fig. 13**

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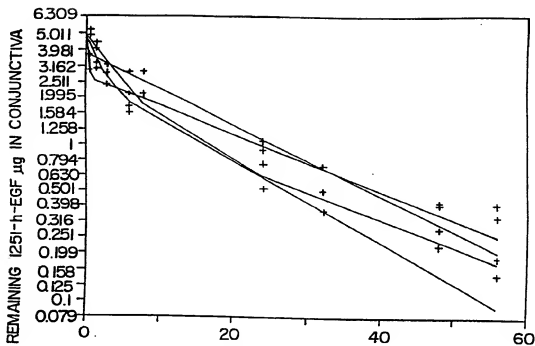


Fig. 14

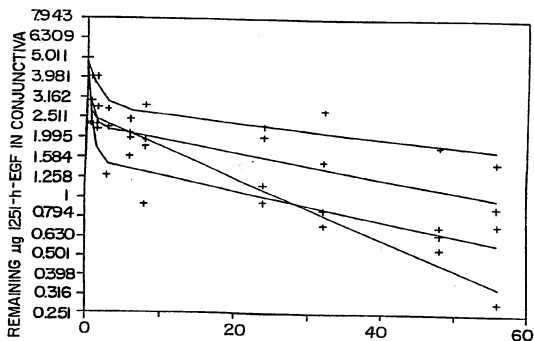


Fig. 15

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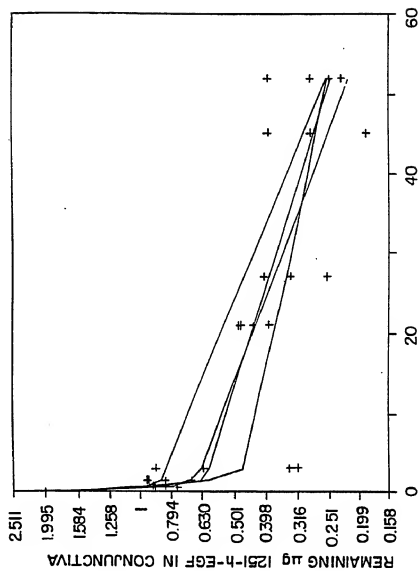
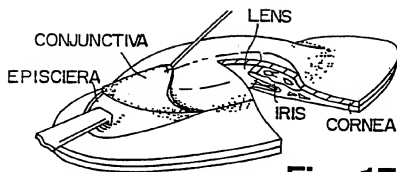
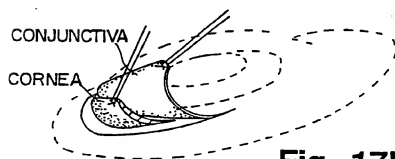
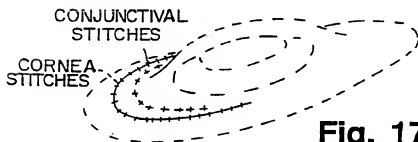
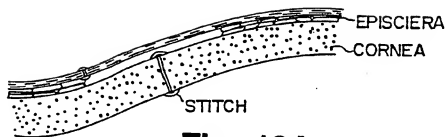
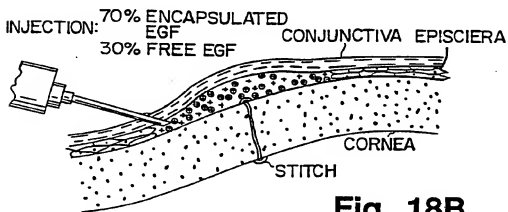
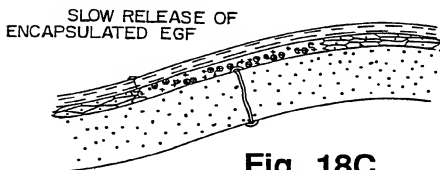


Fig. 16

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**Fig. 17A****Fig. 17B****Fig. 17C**

**Fig. 18A****Fig. 18B****Fig. 18C**

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 90/00918

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC⁵: A 61 K 9/127, 37/36, 9/06

II. FIELDS SEARCHED

Minimum Documentation Searched *

Classification System

Classification Symbols

IPC⁵

A 61 K

Documentation Searched other than Minimum Documentation
to the extent that such Documents are included in the Fields Searched *

III. DOCUMENTS CONSIDERED TO BE RELEVANT*

Category *	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X,Y	STN File Server, (karlsruhe, DE), File Medline, (US. Nat. Library of Medicine) G.L. Brown et al.: "Acceleration of tensile strength of incisions treated with EGF and TGF-beta", AN 89061207, volume 89, & Ann Surg, (1988 Dec) 208 (6) 788- 94 see the abstract --	1-16
X,Y	WO, A, 88/00824 (LIPOSOME TECHN. INC.) 11 February 1988 see page 6, paragraph 2; page 30, line 11 - page 31, line 4; claim 7, claim 9, line 9 & US, A, 4804539 (cited in the application) -- ./.	1-16

* Special categories of cited documents: ¹⁴

"A" document defining the general state of the art which is not
considered to be of particular relevance

"E" earlier document but published on or after the international
filing date

"L" document which may throw doubts on priority claim(s) or
which is cited to establish the publication date of another
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"O" document referring to an oral disclosure, use, exhibition or
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"P" document published prior to the international filing date but
later than the priority date claimed

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cited to understand the principle or theory underlying the
invention

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cannot be considered novel or cannot be considered to
involve an inventive step

"Y" document of particular relevance; the claimed invention
cannot be considered to involve an inventive step when the
document is combined with one or more other such docu-
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in the art.

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IV. CERTIFICATION

Date of the Actual Completion of the International Search

26th June 1990

Date of Mailing of this International Search Report

16 JUL 1990

International Searching Authority

EUROPEAN PATENT OFFICE

Signature of Authorized Officer

[Signature]

Mme N. KUIPER

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, " with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	STN File Server, (Karlsruhe, DE), File Biosis, volume 89, nr 17568, AN 89:292224, Y. Ishii et al.: "Preparation of EGF labeled liposomes and their uptake by hepatocytes", pages 732-736 & Biochem Biophys res commun 160 (2). 1989. 732-736. coden: BBRC9 see the whole abstract --	1,17
A	EP, A, 0162724 (VESTAR) 27 November 1985 see the whole document -----	1-17

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers 18-20 because they relate to subject matter not required to be searched by this Authority, namely:

See PCT-Rule 39.1 (iv): methods for treatment of the human or animal body by surgery or therapy as well as diagnostic methods.

2. ☐ Claim numbers because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ²

This International Searching Authority found multiple inventions in this International application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9000918
SA 35105

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 11/07/90. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A- 8800824	11-02-88	US-A- 4839175	13-06-89
		US-A- 4804539	14-02-89
		EP-A- 0316345	24-05-89
EP-A- 0162724	27-11-85	AU-B- 576363	25-08-88
		AU-A- 4285685	12-12-85
		CA-A- 1263311	28-11-89
		JP-A- 60258109	20-12-85

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(72) Inventors: and

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chützstrasse 102, D-81927 München (DE); WEI-
LAND-WEIBEL, Andrea [DE/DE]; Weidenstrasse
22, D-85662 Hohenbrunn (DE).(74) Agent: MAIWALD, Walter; Maiwald Patentan-
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München (DE).(81) Designated States (national): AE, AG, AL, AM, AT, AU,
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NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG,
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

- With international search report
- Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: A METHOD FOR THE IMPROVEMENT OF TRANSPORT ACROSS ADAPTABLE SEMI-PERMEABLE BARRIERS

(57) Abstract: The invention relates to a method, a kit and a device for controlling the flux of penetrants across an adaptable semi-permeable porous barrier, the method comprising the steps of: preparing a formulation by suspending or dispersing said penetrants in a polar liquid in the form of fluid droplets surrounded by a membrane-like coating of one or several layers, said coating comprising at least two kinds of forms of amphiphilic substances with a tendency to aggregate, said penetrants being able to transport agents through the pores of said barrier or to enable agent permeation through the pores of said barrier after penetrants have entered the pores, selecting a dose amount of said penetrants to be applied on a predetermined area of said barrier to control the flux of said penetrants across said barrier, and applying the selected dose amount of said formulation containing said penetrants onto said area of said porous barrier.

WO 01/01963 A1

A Method for the Improvement of Transport Across
Adaptable Semi-Permeable Barriers

5 The present invention is in the field of administration of drugs and particularly drug delivery across barriers. It more particularly relates to a method for controlling the flux of penetrants across an adaptable, semi-permeable porous barrier. It further relates to a kit and a patch which both enable the drug to be controllably applied.

10 A porous barrier as used herein is any obstacle comprising pores which are too narrow to let the penetrants diffusively pass. This necessarily implies that the penetrants are bigger than the average diameter of such a pore.

15 Some barriers, such as artificial porous membranes, for example ion-track polycarbonate membranes, may have permanent properties, while others are characterised by a possible change of their properties. Most notably the pore size and more rarely the pore density, may change as a function of the surroundings and/or of the flux of the penetrants through the pores in the barrier. The latter can be found with living tissues which are separated by boundaries with such
20 properties, for example, cells and cell organelles.

 The skin is used to further illustrate the basic principle of such a barrier:

25 The maximum barrier properties of the skin reside in the outermost skin region, that is, in the horny layer (*stratum corneum*). This is owing to special chemical and anatomical characteristics of the horny layer, which preclude most efficiently the passage of essentially any material across the skin.

In the stratum corneum, 20-30 consecutive layers of the skin cells (chiefly corneocytes) are organised into columns. These columns are oriented perpendicular to the skin surface, permitting the cells from adjacent columns to overlap laterally and forcing the cells from one layer to be overlaid and packed densely. Intercellular junctions in the horny layer, moreover, are tightly sealed with specialised lipids, chiefly ceramides, which abound in the skin. The skin lipids are also predominantly well packed: typically, they form lipid multilamellae, which are coupled covalently to the neighbouring cell (envelope) membranes. Individual multilamellar stacks that run parallel to the cells surface are joined together with the less well ordered lipid domains. In such domains, the non-ceramide lipids (fatty acids, cholesteryl-sulphate, etc.) prevail.

The skin lipid tendency to self-arrange into densely packed, multilamellar structures is enhanced or even driven, by the hydration or certain ion (e.g. Ca^{2+}) concentration gradients in the skin. This may explain why similar lipid organisation is not observed elsewhere in the body except, with a much lower abundance, in the oral cavity.

Chemical skin permeation enhancers, for example dimethylsulfoxide, promote the diffusion of drugs across the skin by solubilising or extracting some of the intercellular lipids from the barrier. Transcutaneous transport is therefore most efficient in the least tightly packed lipid regions, where hydrophobic pores in the barrier are created most easily. Through such pores sufficiently small and lipophilic agents can diffuse along the transcutaneous concentration gradient(s). The resulting skin permeability is unaffected by the agent concentration, unless the agent acts as an enhancer, but the permeability depends on the concentration and the selection of skin permeation enhancer(s).

However the hydrophobic pores in the skin are not big enough to allow an appreciable transport of large drugs of any kind. Owing to the self-sealing

tendency of the intercellular lipid domains the pores are also rather short lived. The lipophilicity of typical pores in the skin also precludes the transport of hydrophilic, that is, of highly polar, molecules across the organ. Conventional skin permeation enhancement is therefore only useful for the delivery of fatty materials which do not irritate the skin too much, the enhancer-mediated transport and irritation being poorly tolerated by the consumers in many cases.

Therefore to date, permeation based drug delivery through the skin is really successful only for small drugs with a molecular weight below 400 Da. Such drugs can partition into the intercellular lipid matrix in the skin and then diffuse through small hydrophobic pores in the horny layer, first into the skin proper and then further down towards the deep body tissues. The resulting steady state transport is preceded by a short lag-time period, during which the drug traverses the barrier. Transcutaneous transport does not suffer from the first pass effect, however.

The bioavailability of drugs delivered through the skin by such conventional means is typically below 50 %, and often does not even reach 25 % (Hadgraft, 1996; Cevc, 1997).

Large hydrophobic molecules normally cross the skin in negligible quantity only. As already mentioned above this is due to the lack of suitable passages in the skin. Transcutaneous transport of macromolecules therefore chiefly relies on the molecular diffusion through shunts, such as pilosebaceous units. To deliver a bulky and highly polar agent across the skin other methods than those conventionally used are therefore required. For example various skin poration techniques were introduced to create hydrophilic pores in the skin suitable for the purpose (to avoid confusion we will call such hydrophilic pores channels):

5 The simplest, and crudest solution, for making a wide channel through the skin is to eliminate mechanically the skin barrier. For example, to deliver a large, hydrophilic antidiuretic peptide 1-deamino-8-D-arginine vasopressin across the human skin from an occlusive patch the removal of a small piece of epidermis by vacu-suction has been used (Svedman et al., 1996).

10 Further, a most common method for opening a wide channel through the skin is to use an injection needle or mechanical impact(s) (injection; powderjection). Locally restricted skin challenge is also possible. This can be done by local heat application (thermoporation); by using high voltage pulses (>150 V; electroporation); or by acoustic energy, such as ultrasound (few $W\ cm^{-2}$; sonoporation). The resulting channel size depends on the nature and intensity of the skin treatment, but not on the nature or the applied amount of molecules to be transported.

15 Openings or even craters in the skin created by the above mentioned methods heal rather slowly under normal application conditions; the wider the passage, the more so. The skin thus may behave as an adaptable, but slowly recoverable barrier.

20 Even the most commonly used methods for making pores in the skin rely on gadgets plus experience for the proper operation; they also involve skin disinfection to protect the patient. This notwithstanding, their harm and inconvenience is tolerated as long as therapeutic benefit is achieved.

25 The most recent tool for creating hydrophilic passages in those barriers, such as the skin is provided by microscopic barrier penetrants which directly and reversibly open said hydrophilic channels. Such penetrants are independent of external energy source and also do not rely on any gadgets. They are also well tolerated by the skin.

Such penetrants known to date all belong to the class of highly deformable complex droplets (Transfersomes®). Such droplets adapt to the pores of the barrier - which they then cross efficiently - provided that the droplet components and preparation are properly selected and/or optimised. A sufficiently adaptable and hydrophilic droplet can therefore cross the barrier, such as skin, spontaneously. Such hydrophilic channels are opened transiently by the moving penetrant after the latter has adjusted its shape to achieve the goal. This allows the adjustable droplets to act as vehicles for the delivery of various - hydrophilic or hydrophobic - agents across the barrier.

Most useful droplets comprise an aqueous core surrounded by an highly flexible mixed lipid bilayer, which makes the aggregate ultradeformable and superficially highly hydrophilic. Both is required for an efficient transcutaneous transport (Cevc, 1997). Said droplets were demonstrated to transport their mass rather efficiently across the skin under optimum application conditions (Cevc, 1997).

Other types of aggregates (liposomes, niosomes, nanoparticles, microemulsions, etc.) also have been claimed to traverse the skin efficiently but were seldom, if ever, proven really to deliver the associated drugs across the skin in practically meaningful quantities. It is believed that in contrast to the highly deformable droplets (Transfersomes®) the used aggregates are either insufficiently deformable and/or are too unstable to achieve the goal. Conventional aggregates instead act as simple drug reservoirs on the skin: the aggregates, incapable of crossing the barrier, remain on the skin while the drug is released gradually from the 'vehicle' to then probably diffuse through the skin barrier on its own. The main action of conventional drug loaded suspensions is thus to increase the skin barrier hydration and/or to shed the molecules with the skin permeation enhancing capability into the tissue.

Contrary, the composite, ultra deformable lipid droplets (Transfersomes®) deform and then penetrate the skin rather than to coalesce locally. Such aggregates motion across the skin seems to proceed along the natural moisture gradient(s) between the skin cells, which guides the aggregates into the hydrophilic (virtual) channels in the organ.

5

The predecessors of those channels that let highly adaptable droplets pass through the skin are originally so narrow that they only permit evaporation of (rather small) water molecules across the skin. These originally tiny pores (diameter < 0.5 nm) seem to open reversibly, however, when the stress of partial dehydration of a droplet, which is thereby being forced into the channel mouth under non-occlusive conditions, becomes excessive. The strong hydrophilicity and the large mass of the droplet are the factors which maximise the droplets' tendency to move through the skin; however the droplet adaptability is the necessary condition for the success of said motion.

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The movement of the droplets across the skin seems to proceed along the path pursued by the water molecules during the skin passage in the opposite direction. The droplets are thus guided into intercellular regions precisely at the points where the contacts between the above-cited skin sealing lipids are the weakest and the least tight. The corresponding skin region covered with the channels has been estimated to be around 4 % of the total skin area, or less.

20

It is possible to associate small and large, hydrophobic and hydrophilic molecules with ultra deformable and highly adaptable droplet-like aggregates. Using such complex aggregate droplets all types of molecules can thus be delivered across the barrier, such as the stratum corneum.

25

High systemic availabilities of the drug transported are typically achieved. Relative efficiency of the transport across the skin exceeds 50 %, in most cases (Cevc et al., 1996). The steady state is reached within few hours, by and large (Cevc et al., 1998).

5

It has already been proven that the skin barrier recovers fully after those droplets have been eliminated from the skin surface. In contrast, the channels created by other means, such as ultrasound remain open for at least 20 hours. In fact, they are not resealed properly before 2 days, even when relatively weak therapeutic ultrasound is used. Stronger perturbation causes more persistent skin damage (Mitragotri et al., 1995). (In the extreme case, when the barrier is eliminated by vacu-suction, the skin does not recover fully until after of 8 weeks.)

10

The precise size distribution of the channels in the skin, through which highly deformable droplets migrate spontaneously across the stratum corneum, is as yet unknown. It is probable, however, that it is asymmetric. The average width, that is, the distribution maximum has been estimated to be 20-30 nm under typically used application conditions. The skewed distribution could result from the existence of two quantitatively different but qualitatively similar intercellular transport routes across the skin (Schätzlein & Cevc, 1998) which together form the family of transcutaneous pathways.

15

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The first, inter-cluster pathway leads between the groups of corneocytes. It represents the high-end tail of channel-size distribution and typically starts at the bottom of inter-cluster gorges. From here, it follows the dense material filling such gorge and offers the lowest resistance to penetration at the junctions where several clusters meet.

25

The second, intra-cluster pathway leads between the individual corneocytes in each cluster of corneocytes. This route typically proceeds along the lipid layers surface. In the projection over the outer third of the stratum corneum, the inter-corneocyte pathway resembles an interwoven three-dimensional network including all the cells in the organ. (Schätzlein & Cevc, 1998).

The above mentioned distinctions are quantitative in nature. No doubt exists that transcutaneous channels with the exception of pilosebaceous units are resistant to the passage of non-deformable, large aggregates.

Channel properties are also sufficiently constant to reveal little inter-site, inter-individual, inter-species or inter-carrier variability. According to the prior art, the relative bio-availability of different drugs in the blood after an epicutaneous administration in highly adaptable droplets (Transfersomes®) is fairly constant (Cevc, 1997). Pore distribution depends little on the nature of the penetrant or the drug. The same has been implied for the dose dependence, which was concluded to affect merely the depth of penetrant and drug distribution. Small dose per area was found to favour the local (superficial) retention whereas a large dose per area was shown to ensure a relatively great systemic availability.

Surprisingly, and contrary to the above-mentioned conclusion, we have now found out that changing the applied dose above a certain threshold and in sufficiently wide range not only affects the drug/penetrant distribution, but also determines the rate of penetrant transport across the barrier.

Our new and unexpected finding provides means for controlling the rate of transcutaneous drug delivery whenever highly dcformable carriers are used on the barrier; it also provides the basis for better, i.e. more rational, design of the delivery device. There will especially be profit for the development of cutaneous

patches suitable for the use in combination with highly adaptable carriers (Transfersomes®). Improved therapy and higher commercial value of the products should be the consequence.

5 It stands to reason that the observed new effect reflects the widening of channels in the barrier, but the applicant does not wish to be bound to this hypothesis. The newly found dosage-dependent pore widening is probably different for various transcutaneous channels: the originally narrower pores probably change more than the relatively wide (e.g. inter-cluster) channels. The effect of relative channel
10 size, that is, of channel vs. penetrant size ratio, suggests that it will take much longer time to bring certain penetrants quantity through narrow than through wide channels.

If the channels act as transported mass discriminators, and adjust their width to the
15 flux requirement, the narrow channels will persist much longer in their original, high penetration resistance state than the wide channels. However, after having responded to the multi-penetrant passage by increasing their width such channels will start to behave as the originally wider channels. Multiple adjustments are possible but only to certain upper limit.

20 Another potentially important factor acting in the same direction is the skin surface hydration, which is prone to increase with enlargement of the topically administered dosage. In either case, the average width and the size distribution of channels in the skin will shift towards greater values with increasing applied
25 dosage. This then will result in higher final transcutaneous flux.

For the avoidance of doubt, all pertinent information, definitions and lists from the previous patent applications of the same applicant are incorporated herein by reference.

Kits and more particularly devices for administering drugs through a barrier such as skin or mucosa have also already been described. These devices can typically be divided into matrix systems and liquid reservoir systems.

5

Container-type reservoirs are often formed as a pocket between the backing layer and a rate controlling membrane through which the drug passes to the skin. The pressure sensitive adhesive layer normally underlies the membrane and the drug also passes through it on its way to the skin.

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As mentioned above it is customary to prepare reservoir type patches for transdermal drug delivery with a backing membrane and a rate controlling membrane (Ogiso, T., Y. Ito, et al. (1989). "Membrane-controlled transdermal therapeutic system containing clonazepam and anticonvulsant activity after its application." *Chem Pharm Bull (Tokyo)* 37, 446-9; Ito, Y., T. Ogiso, et al. (1993). "Percutaneous absorption of acetaminophen from a membrane controlled transdermal system and prediction of the disposition of the drug in rats" *Biol. Pharm. Bull* 16, 583-8)

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A number of reservoir type systems have been described.

US-Patent No. 829,224 to Chang et al., for instance, discloses a device with a reservoir that is defined by a backing layer and a drug-permeable membrane layer. A ring-shaped layer made of an adhesive is peripheral to the reservoir. A peelable liner layer underlies the membrane. A second peelable layer, the release liner, underlies the entire assembly. A first heat seal connects the backing layer and the membrane and surrounds the reservoir. A second heat seal concentric about the first heat seal connects the backing layer and the release liner. The second heat seal is broken when the release liner is removed. The device may include an inner

25

liner that underlies the membrane and portions of the backing layer. This inner liner is removed following removal of the release liner so that the membrane is exposed.

5 U.S.-Patent Nr. 4,983,395 to Chang et al., relates to another device with a backing layer and a membrane layer that define a reservoir. A peelable inner liner underlies the reservoir and portions of the backing and membrane layers outside the periphery of the reservoir. An adhesive layer underlies the inner liner and remaining portions of the backing and membrane layers. A peelable release liner
10 underlies the adhesive layer. A first heat seal connects the backing and membrane layers on the periphery of the reservoir. A second heat seal underlies the first heat seal and connects the membrane and the inner liner. In use, the release liner and inner liner are peeled away to expose the undersurfaces of the membrane and adhesive layers prior to placement of the device onto the skin or mucosa.

15 PCT-Application W096-19205 to Theratech, Inc., discloses a device for administering an active agent to the skin or mucosa of an individual comprising a laminated composite of an adhesive overlay, a backing layer underlying the central portion of the adhesive overlay, an active agent-permeable membrane, the backing
20 layer and membrane defining a reservoir that contains a formulation of the active agent, a peel seal disc underlying the active agent-permeable membrane, a heat seal about the periphery of the peel seal disc, the active agent-permeable membrane and the backing layer and a removable release liner underlying the exposed overlay and peel seal disc. The adhesive layer is above and peripheral to
25 the path of the active agent to the skin or mucosa and is protected from degradation by the components of the reservoir by a multiplicity of heat seals. The peel seal disc protects against release of the active agent-containing reservoir and the release liner protects the adhesive from exposure to the environment prior to use.

US-Patent No. 5,202,125 to Theratech, Inc., describes a transdermal delivery system for delivery of nitroglycerin which deliver the drug at enhanced transdermal fluxes. The systems include, in addition to nitroglycerin, a permeation enhancer which is either a sorbitan ester, a C8-C22 aliphatic alcohol, or a mixture thereof. Methods for administering nitroglycerin using such permeation enhancers are also disclosed.

WO90-11065 to Theratech, Inc., discloses a transdermal drug delivery device comprising a drug formulation containing reservoir defined by a backing layer and a drug-permeable membrane layer, a peelable inner liner that underlies the reservoir and a portion of the backing/membrane outwardly of the reservoir periphery, an adhesive layer that underlies the inner liner and outwardly extending portions of the membrane/backing layers, and a peelable release liner layer that underlies the adhesive layer with a first permanent heat seal between the backing and the membrane about the perimeter of the reservoir and another peelable (impermeant) heat seal between the membrane and the inner liner underlying the first permanent heat seal, the heat seals and peelable barrier layer providing barriers that isolate the drug formulation from the adhesive.

Depending on the features to be achieved, backing films are either occlusive or permeable and commonly are derived from synthetic polymers, such as polyester, polyethylene, polyvinylidene chloride (PVDC), polyurethane or natural polymers, such as cotton, wool, etc. It is possible to use nonporous, microporous, such as polypropylene or polyethylene or also macroporous woven and nonwoven materials as a backing layer in transdermal patches. The backing layers are generally selected from these materials depending on the active agent to be delivered.

Occlusive backings in classical TTS (transdermal transport systems) tend to promote higher deposition and a higher rate of permeation of the active or inactive ingredients into the skin compared to non-occlusive backing. Occlusive backings are e.g. desirable to enhance the delivery of steroids to the lower layers of the epidermis to treat inflammation and dermatoses. Examples are Actiderm® (dermatological patch) or Cordran® (tape and patch).

Semi-occlusive films, such as polyurethanes and polyolefin copolymers, and non-occlusive woven and nonwoven fiber-based materials, such as cotton and polyester, allow water vapor transmission from the skin surface and from the patch. These semi-occlusive or non-occlusive materials are rarely used as backing materials in TTS. Thicker non-occlusive backings were only desirable for corn and callus removal products since the active agent needs only to be delivered to the outer layers of the stratum corneum. The non-occlusive woven and nonwoven materials used in many of these products mainly serve as a protective cushion.

Rate controlling membranes usually used in commercial TTS are thin (26 – 78 µm) nonporous ethylene vinyl acetate films, such as Transderm-Nitro® (Ciba-Geigy and ZAFFARONI) Duragesic®, Estraderm®, and Estragel®. Moreover, thin (26 – 78 µm) microporous films of polyethylene, such as Transderm-Scop®, Catapres® are used as rate controlling membrane in multilaminate solid state reservoir patches or in liquid reservoir TTS. Further examples for such microporous PE-membranes are β-Estro® and Androderm®. These membranes usually serve to limit the rate of diffusion of the drug onto and through the skin.

As already described above Transfersomes® are able to mediate agent or drug delivery through the skin due to the hydration gradient across the biological barrier. In contrary to customary transdermal transport systems, wherein the agent mediation commonly depends on classical Fick's law of diffusion, therapeutic

systems suitable for Transfersomes® and useful for the method of the present invention must fulfill different criteria.

5 It is also problematic that Transfersome®-mediated drug delivery through the skin from a patch is hindered if an occlusive backing material is used. The use of an occlusive membrane as backing layer causes an increased Transfersomes® hydration, since e.g. vapors cannot leak from the patch. Accordingly the hydration gradient and therefore the driving force for the Transfersome® transport is dramatically lowered.

10 Another problem is that many of the non-occlusive woven and nonwoven backings, which customary serve as a protective cushion, retain the Transfersomes® due to adsorption and trapping of lipids and proteins in the fibrous structure.

15 Moreover, any classical microporous and non-porous rate-controlling membranes having a pore size of smaller than about 20 nm may interfere with the passage of Transfersomes® through the pores due to size exclusion.

20 It is obvious to someone skilled in the art that the known transdermal patches having conventional backing and rate controlling membranes are not suitable for the mediation of Transfersomes® according to the present invention. The same applies to matrix-type patches.

25 In matrix-type transdermal patches are those in which the drug is contained in and released from a polymer matrix. The matrix is typically made of a pressure sensitive adhesive and defines the basal surface of the patch (i.e. the surface affixed to the skin).

A number of matrix type systems have been described.

US-Patent No. 5,460,820 to Theratech, Inc., discloses a method of providing testosterone replacement therapy to a woman in need of such therapy comprising
5 applying a testosterone-delivering patch to the skin of said woman which patch transdermally delivers 50 to 500 $\mu\text{g/day}$ testosterone to the woman. The skin patch comprises a laminated composite of a backing layer and a matrix layer comprising a solution of testosterone in a polymeric carrier, said matrix layer providing a sufficient daily dose of testosterone to provide said therapy.

10 US-Patent No. 5,783,208 to Theratech, Inc., discloses a matrix-type transdermal patch for coadministering estradiol and another steroid wherein the matrix is composed of a N-vinyl-2-pyrrolidone-containing acrylic copolymer pressure sensitive adhesive, estradiol the other steroid, and optionally a permeation
15 enhancer, and the respective fluxes of estradiol and the other steroid from the matrix are independent of the respective concentrations of the other steroid and estradiol in the matrix.

All pertinent information, definitions and lists from the patents and patent
20 applications of the US-company Theratech, Inc. are expressively incorporated herein by reference.

As mentioned above, it is customary to prepare reservoir type patches for transdermal drug delivery with a backing membrane and a rate controlling
25 membrane. These membranes form typically one compartment, which contains the corresponding formulation. This can be a - mostly alcoholic or aqueous - solution, an aqueous suspension or a gel which contains gel forming polymers. Parameters as chemical and physical stability, viscosity, concentrations of active ingredient(s) and excipients are not critical with respect to commercial one-compartment

reservoir-types, since the currently most active ingredients (drugs) are stable, low-molecular-weight substances (nicotine, fentanyl, estradiol, scopolamine and others), which commonly do not interfere with e.g. additional ingredients such as antioxidants, stabilizers, cosolvents or penetration enhancers.

5

As already mentioned, the Transfersome®-mediated drug delivery through barriers clearly differs from customary drug delivery through the skin. While it is not possible administering high molecular drugs by transdermal patches known in the art, Transfersomes® in principle are suitable carriers for a drug of high molecular weight such as peptides (e.g. insulin) and proteins (serum albumin). It is clear to someone skilled in the art that problems may arise if e.g. labile proteins are mixed with interfering or destabilizing ingredients over an extended storage period in customary one-compartment patches.

15

In many cases sufficient stabilities of all ingredients are not achievable within one compartment. For example Transfersome®-forming phospholipids are most stable at pH 6.5, while proteins may have other pH values of optimal stability (e.g. Interferon- α -2b at pH = 7.4 or pH = 3). Therefore, it would be necessary to keep said substances in different media if stored over an extended time period. For example, Transfersomes of type-T are formulated and stable in phosphate-buffer, while hepatocyte growth factor (HGF) is stable in citrate-buffer. Moreover, commonly organic (co-)solvents are used to introduce antioxidants such as BHT into lipid aggregates. Said (co-)solvents may contribute to reduced solubility of the proteins as they lower the bulk dielectricity constant, thus reducing electrostatic repulsion. This may lead to uncontrolled, at least unwanted, aggregation and denaturation of the proteins.

25

It is an important object of the present invention to control the flux of highly deformable penetrants (Transfersomes®) across an adaptable semi-permeable

porous barrier, such as the skin of a human or animal body or a plant. It is another object of the present invention to control the flux of highly deformable penetrants (Transfersomes®) across an adaptable semi-permeable porous barrier in using a kit or transdermal transport system which enables the formulation to be applied at the selected dose per area. It is a further object of the present invention to provide a reservoir-type transdermal patch suitable for the Transfersome®-mediated agent or drug delivery through the intact skin. Another object of the present invention is the provision of a long term stable multicompartment reservoir-type transdermal patch, which comprises separate compartments and is suitable for the Transfersome®-mediated agent or drug delivery through the intact skin.

- According to the present invention this is achieved by a method for controlling the flux of penetrants across an adaptable semi-permeable porous barrier comprising the steps of:
- 15 – preparing a formulation by suspending or dispersing said penetrants in a polar liquid in the form of fluid droplets surrounded by a membrane-like coating of one or several layers, said coating comprising at least two kinds or forms of amphiphilic substances with a tendency to aggregate, provided that
 - 20 – said at least two substances differ by at least a factor of 10 in solubility in said polar liquid,
 - and / or said substances when in the form of homo-aggregates (for the more soluble substance) or of hetero-aggregates (for any combination of both said substances) have a preferred average diameter smaller than the diameter of homo-aggregates containing merely the less soluble substance,
 - 25 – and / or the more soluble substance tends to solubilise the droplet and the content of such substance is to up to 99 mol-% of solubilising concentration or else corresponds to up to 99 mol-% of the saturating concentration in the unsolubilised droplet, whichever is higher;

- and / or the presence of the more soluble substance lowers the average elastic energy of the membrane-like coating to a value at least 5 times lower, more preferably at least 10 times lower and most preferably more than 10 times lower, than the average elastic energy of red blood cells or of phospholipid bilayers with fluid aliphatic chains,
- said penetrants being able to transport agents through the pores of said barrier or to enable agent permeation through the pores of said barrier after penetrants have entered the pores,
- selecting a dose amount of said penetrants to be applied on a predetermined area of said barrier to control the flux of said penetrants across said barrier, and
- applying the selected dose amount of said formulation containing said penetrants onto said area of said porous barrier.

Preferrably the flux of penetrants across said barrier is increased by enlarging the applied dose amount of said penetrants.

It then is preferred if the pH of the formulation is between 3 and 10, more preferably is between 4 and 9, and most preferably is between 5 and 8.

- According to another preferred feature of the present invention the formulation containing the penetrants comprises:
- at least one thickening agent in an amount to increase the formulation viscosity to maximally 5 Nm/s, more preferably up to 1 Nm/s, and most preferably up to 0.2 Nm/s, so that formulation spreading-over, and drug retention at the application area is enabled,
 - and / or at least one antioxidant in an amount that reduces the increase of oxidation index to less than 100 % per 6 months, more preferably to less than 100 % per 12 months and most preferably to less than 50 % per 12 months

- 5 — and / or at least one microbicide in an amount that reduces the bacterial count of 1 million germs added per g of total mass of the formulation to less than 100 in the case of aerobic bacteria, to less than 10 in the case of entero-bacteria, and to less than 1 in the case of *Pseudomonas aeruginosa* or *Staphylococcus aureus*, after a period of 4 days.

10 It then is preferred if said at least one microbicide is added in an amount that reduces the bacterial count of 1 million germs added per g of total mass of the formulation to less than 100 in the case of aerobic bacteria, to less than 10 in the case of entero-bacteria, and to less than 1 in the case of *Pseudomonas aeruginosa* or *Staphylococcus aureus*, after a period of 3 days, and more preferably after a period of 1 day.

15 It then is also preferred if said thickening agent is selected from the class of pharmaceutically acceptable hydrophilic polymers, such as partially etherified cellulose derivatives, like carboxymethyl-, hydroxyethyl-, hydroxypropyl-, hydroxypropylmethyl- or methyl-cellulose; completely synthetic hydrophilic polymers such as polyacrylates, polymethacrylates, poly(hydroxyethyl)-, poly(hydroxypropyl)-, poly(hydroxypropylmethyl)methacrylates,
20 polyacrylonitriles, methallyl-sulphonates, polyethylenes, polyoxiethylenes, polyethylene glycols, polyethylene glycol-lactides, polyethylene glycol-diacrylates, polyvinylpyrrolidones, polyvinyl alcohols, poly(propylmethacrylamides), poly(propylene fumarate-co-ethylene glycols), poloxamers, polyaspartamides, (hydrazine cross-linked) hyaluronic acids, silicones; natural gums comprising
25 alginates, carrageenans, guar-gums, gelatines, tragacanth, (amidated) pectins, xanthans, chitosan collagens, agaroses; mixtures and further derivatives or co-polymers thereof and / or other pharmaceutically, or at least biologically, acceptable polymers.

Preferrably the concentration of said polymer is chosen to be in the range between 0.01 w- % and 10 w- %, more preferably in the range between 0.1 w- % and 5 w- %, even more preferably in the range between 0.25 w- % and 3.5 w- % and most preferably in the range between 0.5 w- % and 2 w- %.

5 Further it is preferred that said anti-oxidant is selected from synthetic phenolic antioxidants, such as butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT) and di-tert-butylphenol (LY178002, LY256548, HWA-131, BF-389, CI-986, PD-127443, E-51119, BI-L-239XX, etc.), tertiary butylhydroquinone (TBHQ),
10 propyl gallate (PG), 1-O-hexyl-2,3,5-trimethylhydroquinone (HTHQ); aromatic amines (such as diphenylamine, p-alkylthio-o-anisidine, ethylenediamine derivatives, carbazol, tetrahydroindenoindol); phenols and phenolic acids (such as guaiacol, hydroquinone, vanillin, gallic acids and their esters, protocatechuic acid, quinic acid, syringic acid, ellagic acid, salicylic acid, nordihydroguaiaretic acid
15 (NDGA), eugenol); tocopherols (including tocopherols (alpha, beta, gamma, delta) and their derivatives, such as tocopheryl-acylate (e.g. -acetate, -laurate, myristate, -palmitate, -oleate, -linoleate, etc., or any other suitable tocopheryl-lipoate), tocopheryl-POE-succinate; trolox and corresponding amide- and thiocarboxamide analogues; ascorbic acid and its salts, isoascorbate, (2 or 3 or 6)-o-alkylascorbic
20 acids, ascorbyl esters (e.g. 6-o-lauroyl, myristoyl, palmitoyl-, oleoyl, or linoleoyl-L-ascorbic acid, etc.); non-steroidal anti-inflammatory agents (NSAIDs), such as indomethacin, diclofenac, mefenamic acid, flufenamic acid, phenylbutazone, oxyphenbutazone acetylsalicylic acid, naproxen, diflunisal, ibuprofen, ketoprofen, piroxicam, penicillamine, penicillamine disulphide,
25 primaquine, quinacrine, chloroquine, hydroxychloroquine, azathioprine, phenobarbital, acetaminophen); aminosalicylic acids and derivatives; methotrexate, probucol, antiarrhythmics (e.g. amiodarone, aprindine, asocainol), ambroxol, tamoxifen, b-hydroxytamoxifen; calcium antagonists (such as nifedipine, nisoldipine, nimodipine, nicardipine, nilvadipine), beta-receptor

blockers (e.g. atenolol, propranolol, nebivolol); sodium bisulphite, sodium metabisulphite, thiourea; chelating agents, such as EDTA, GDTA, desferral; endogenous defence systems, such as transferrin, lactoferrin, ferritin, cearyluloplasmin, haptoglobin, haemopexin, albumin, glucose, ubiquinol-10;

5 enzymatic antioxidants, such as superoxide dismutase and metal complexes with a similar activity, including catalase, glutathione peroxidase, and less complex molecules, such as beta-carotene, bilirubin, uric acid; flavonoids (e.g. flavones, flavonols, flavonones, flavanols, chacones, anthocyanins), N-acetylcystein, mesna, glutathione, thiohistidine derivatives, triazoles; tannins, cinnamic acid,

10 hydroxycinnamic acids and their esters (e.g. coumaric acids and esters, caffeic acid and their esters, ferulic acid, (iso-) chlorogenic acid, sinapic acid); spice extracts (e.g. from clove, cinnamon, sage, rosemary, mace, oregano, allspice, nutmeg); carnosic acid, carnosol, carnosolic acid; rosmarinic acid, rosmarindiphenol, gentisic acid, ferulic acid; oat flour extracts, such as avenanthramide 1 and 2;

15 thioethers, dithioethers, sulfoxides, tetraalkylthiuram disulphides; phytic acid, steroid derivatives (e.g. U74006F); tryptophan metabolites (e.g. 3-hydroxykynurenine, 3-hydroxyanthranilic acid), and organochalcogenides, or else is an oxidation suppressing enzyme.

20 Then, the concentration of BHA or BHT is often chosen to be between 0.001 and 2 w-%, more preferably is between 0.0025 and 0.2 w-%, and most preferably is between 0.005 and 0.02 w-%, of TBHQ and PG is between 0.001 and 2 w-%, more preferably is between 0.005 and 0.2 w-%, and most preferably is between 0.01 and 0.02 w-%, of tocopherols is between 0.005 and 5 w-%, more preferably

25 is between 0.01 and 0.5 w-%, and most preferably is between 0.05 and 0.075 w-%, of ascorbic acid esters is between 0.001 and 5, more preferably is between 0.005 and 0.5, and most preferably is between 0.01 and 0.15 w-%, of ascorbic acid is between 0.001 and 5, more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01 and 0.1 w-%, of sodium bisulphite or sodium

metabisulphite is between 0.001 and 5, more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01-0.15 w-%, of thiourea is between 0.0001 and 2 w-%, more preferably is between 0.0005 and 0.2, and most preferably is between 0.001-0.01 w-%, most typically 0.005 w-%, of cystein is
5 between 0.01 and 5, more preferably is between 0.05 and 2 w-%, and most preferably is between 0.1 and 1.0 w-%, most typically 0.5 w-%, of monothioglycerol is between 0.01 and 5 w-%, more preferably is between 0.05 and 2 w-%, and most preferably is between 0.1-1.0 w-%, most typically 0.5 w-%, of NDGA is between 0.0005-2 w-%, more preferably is between 0.001-0.2 w-%,
10 and most preferably is between 0.005-0.02 w-%, most typically 0.01 w-%, of glutathione is between 0.005 and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most preferably is between 0.05 and 0.2 w-%, most typically 0.1 w-%, of EDTA is between 0.001 and 5 w-%, even more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01 and 0.2 w-%, most
15 typically between 0.05 and 0.975 w-%, of citric acid is between 0.001 and 5 w-%, even more preferably is between 0.005 and 3 w-%, and most preferably is between 0.01-0.2, most typically between 0.3 and 2 w-%.

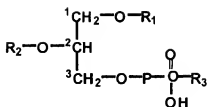
Furthermore it is preferred if said microbicide is selected amongst short chain
20 alcohols, such as ethyl and isopropyl alcohol, chlorbutanol, benzyl alcohol, chlorbenzyl alcohol, dichlorbenzylalcohol; hexachlorophene; phenolic compounds, such as cresol, 4-chloro-m-cresol, p-chloro-m-xylenol, dichlorophene, hexachlorophene, povidon-iodine; parabens, especially alkyl-paraben, such as methyl-, ethyl-, propyl-, or butyl-paraben, benzyl-paraben;
25 acids, such as sorbic acid, benzoic acid and its salts; quaternary ammonium compounds, such as alkonium salts, e.g. benzalkonium salts, especially the chlorides or bromides, cetrimonium salts, e.g. the bromide; phenoalkecinium salt, such as phenododecinium bromide, cetylpyridinium chloride or other such salts; mercurium compounds, such as phenylmercuric acetate, borate, or nitrate,

thiomersal; chlorhexidine or its gluconate; antibioticly active compounds of biological origin, or a mixture thereof.

5 Preferrably the bulk concentration of short chain alcohols in the case of ethyl, propyl, butyl or benzyl alcohol is up to 10 w-%, more preferably is up to 5 w-%, and most preferably is in the range between 0.5-3 w-%, and in the case of chlorobutanol is in the range between 0.3-0.6 w-%; bulk concentration of parabens, especially in the case of methyl paraben is in the range between 0.05-0.2 w-%, and in the case of propyl paraben is in the range between
10 0.002-0.02 w-%; bulk concentration of sorbic acid is in the range between 0.05-0.2 w-%, and in the case of benzoic acid is in the range between 0.1-0.5 w-%; bulk concentration of phenols, triclosan, is in the range between 0.1-0.3 w-%, and bulk concentration of chlorhexidine is in the range between 0.01-0.05 w-%.

15 It is preferred that the less soluble amongst the aggregating substances is a lipid or lipid-like material, especially a polar lipid, whereas the substance which is more soluble in the suspending liquid and which lowers the average elastic energy of the droplet is a surfactant or else has surfactant-like properties and / or is a form of said lipid or lipid-like material which is comparably soluble as said surfactant or
20 the surfactant-like material.

 Preferrably the lipid or lipid-like material is a lipid or a lipid from a biological source or a corresponding synthetic lipid or any of its modifications, said lipid preferably belonging to the class of pure phospholipids corresponding to the
25 general formula



- where R₁ and R₂ is an aliphatic chain, typically a C₁₀₋₂₀-acyl, or -alkyl or partly unsaturated fatty acid residue, in particular, an oleoyl-, palmitoeloyl-, elaidoyl-, linoleyl-, linolenyl-, linolenoyl-, arachidoyl-, vacciny-, lauroyl-, myristoyl-, palmitoyl-, or stearoyl chain; and where R₃ is hydrogen, 2-trimethylamino-1-ethyl, 2-amino-1-ethyl, C₁₋₄-alkyl, C₁₋₅-alkyl substituted with carboxy, C₂₋₅-alkyl substituted with hydroxy, C₂₋₅-alkyl substituted with carboxy and hydroxy, or C₂₋₅-alkyl substituted with carboxy and amino, inositol, sphingosine, or salts of said substances, said lipid comprising also glycerides, isoprenoid lipids, steroids, sterines or sterols, of sulphur- or carbohydrate-containing lipids, or any other bilayer-forming lipids, in particular half-protonated fluid fatty acids, said lipid is selected from the group comprising phosphatidylcholines, phosphatidylethanolamines, phosphatidylglycerols, phosphatidylinositols, phosphatidic acids, phosphatidylserines, sphingomyelins or other sphingophospholipids, glycosphingolipids (including cerebrosides, ceramidepolyhexosides, sulphatides, sphingoplasmalogens), gangliosides and other glycolipids or synthetic lipids, in particular with corresponding sphingosine derivatives, or any other glycolipids, whereby two similar or different chains can be ester-groups-linked to the backbone (as in diacyl and dialkenoyl compound) or be attached to the backbone with ether bonds, as in dialkyl-lipids.

The surfactant or surfactant-like material preferably is a nonionic, a zwitterionic, an anionic or a cationic surfactant, especially a fatty-acid or -alcohol, an alkyl-

- tri/di/methyl-ammonium salt, an alkylsulphate salt, a monovalent salt of cholate, deoxycholate, glycocholate, glycodeoxycholate, taurodeoxycholate, taurocholate, etc., an acyl- or alkanoyl-dimethyl- aminoxide, esp. a dodecyl- dimethyl- aminoxide, an alkyl- or alkanoyl-N-methylglucamide, N- alkyl-N,N-dimethylglycine, 3-(acyldimethylammonio)-alkanesulphonate, N-acyl-
- 5 sulphobetaine, a polyethylene-glycol-octylphenyl ether, esp. a nonaethylene-glycol-octylphenyl ether, a polyethylene-acyl ether, esp. a nonaethylen-dodecyl ether, a polyethylene-glycol-isoacyl ether, esp. a octaethylene-glycol-isotridecyl ether, polyethylene-acyl ether, esp. octaethylenedodecyl ether, polyethylene-
- 10 glycol-sorbitane-acyl ester, such as polyethyleneglycol-20-monolaurate (Tween 20) or polyethyleneglycol-20-sorbitan-monooleate (Tween 80), a polyhydroxyethylene-acyl ether, esp. polyhydroxyethylene- lauryl, -myristoyl, -cetylstearyl, or -oleoyl ether, as in polyhydroxyethylene-4 or 6 or 8 or 10 or 12, etc., -lauryl ether (as in Brij series), or in the corresponding ester, e.g. of polyhydroxyethylen-8-stearate
- 15 (Myrj 45), -laurate or -oleate type, or in polyethoxylated castor oil 40, a sorbitane-monoalkylate (e.g. in Arlacel or Span), esp. sorbitane-monolaurate, an acyl- or alkanoyl-N-methylglucamide, esp. in or decanoyl- or dodecanoyl-N-methylglucamide, an alkyl-sulphate (salt), e.g. in lauryl- or oleoyl-sulphate, sodium deoxycholate, sodium glycodeoxycholate, sodium oleate, sodium taurate,
- 20 a fatty acid salt, such as sodium elaidate, sodium linoleate, sodium laurate, a lysophospholipid, such as n-octadecylene(=oleoyl)-glycerophosphatidic acid, -phosphorylglycerol, or -phosphorylserine, n-acyl-, e.g. lauryl or oleoyl-glycerophosphatidic acid, -phosphorylglycerol, or -phosphorylserine, n-tetradecyl-glycero-phosphatidic acid, -phosphorylglycerol, or - phosphorylserine, a
- 25 corresponding palmitoeloyl-, elaidoyl-, vaccenyl-lysophospholipid or a corresponding short-chain phospholipid, or else a surface-active polypeptide.

According to a preferred feature of the present invention, the average diameter of the penetrant is between 30 nm and 500 nm, more preferably between 40 nm and

250 nm, even more preferably between 50 nm and 200 nm and particularly preferably between 60 nm and 150 nm.

5 It is another preferred feature of the present invention that the total dry weight of droplets in a formulation is 0.01 weight-% (w-%) to 40 w-% of total formulation mass, more preferably is between 0.1 w-% and 30 w-%, and most preferably is between 0,5 w-% and 20 w-%.

10 It is preferred that the total dry weight of droplets in a formulation is selected to increase the formulation viscosity to maximally 200 mPas, more preferably up to 40 mPas, and most preferably up to 8 mPas.

15 According to the present invention is preferred if at least one edge-active substance or surfactant and/or at least one amphiphilic substance, and / or at least one hydrophilic fluid and the agent are mixed, if required separately, to form a solution, the resulting (partial) mixtures or solutions are then combined subsequently to induce, preferably by action of mechanical energy such as shaking, stirring, vibrations, homogenisation, ultrasonication, shearing, freezing and thawing, or filtration using convenient driving pressure, the formation of
20 penetrants that associate with and / or incorporate the agent

25 Preferably this amphiphilic substances are dissolved in volatile solvents, such as alcohols, especially ethanol, or in other pharmaceutically acceptable organic solvents, such as ethanol, 1- and 2-propanol, benzyl alcohol, propylene glycol, polyethylene glycol (molecular weight: 200-400 D) or glycerol, other pharmaceutically acceptable organic solvents, such as undercooled gas, especially supercritical CO₂, which are then removed, especially by evaporation or dilution, prior to making the final preparation.

According to the present invention the formation of said penetrants preferably is induced by the addition of required substances into a fluid phase, evaporation from a reverse phase, by injection or dialysis, if necessary under the influence of mechanical stress, such as shaking, stirring, especially high velocity stirring, vibrating, homogenising, ultrasonication, shearing, freezing and thawing, or filtration using convenient, especially low (1 MPa) or intermediate (up to 10 MPa), driving pressure.

Then the formation of said penetrants preferably is induced by filtration, the filtering material having pores sizes between 0.01 μm and 0.8 μm , more preferably between 0.02 μm and 0.3 μm , and most preferably between 0.05 μm and 0.15 μm , whereby several filters may be used sequentially or in parallel.

According to the invention said agents and penetrants preferably are made to associate, at least partly,

- after the formation of said penetrants, e.g. after injecting a solution of the drug in a pharmaceutically acceptable fluid, such as ethanol, 1- and 2-propanol, benzyl alcohol, propylene glycol, polyethylene glycol (molecular weight: 200-400 D) or glycerol into the suspending medium,
- simultaneously with penetrant formation, if required using the drug co-solution and, at least some, penetrant ingredients.

It is preferred if said penetrants, with which the agent is associated are prepared immediately before the application of the formulation, if convenient, from a suitable concentrate or a lyophilisate.

The formulation according to the invention preferably is applied by spraying, smearing, rolling or sponging on the application area, in particular by using a metering sprayer, spender, roller, sponge or a non-occlusive patch, as appropriate.

It is preferred if the barrier is a part of a mammalian body and / or a plant and preferably is skin and / or at least partly keratinised endothelium and / or nasal or any other mucosa.

5

The area dose of said penetrant then preferably is between 0.1 mg per square centimetre (mg cm^{-2}) and 40 mg cm^{-2} , more preferably is between 0.25 mg cm^{-2} and 30 mg cm^{-2} and even more preferably is between 0.5 mg cm^{-2} and 15 mg cm^{-2} , in the case that the penetrant is applied on said skin and / or said at least partly keratinised endothelium.

10

The area dose of said penetrant then preferably is between 0.0001 mg per square centimetre (mg cm^{-2}) and 0.1 mg cm^{-2} , more preferably is between 0.0005 mg cm^{-2} and 0.05 mg cm^{-2} and even more preferably is between 0.001 mg cm^{-2} and 0.01 mg cm^{-2} , in the case that the penetrant is applied on plant body, plant leaves or plant needles.

15

The area dose of said penetrant then preferably is between 0.05 mg per square centimetre (mg cm^{-2}) and 20 mg cm^{-2} , more preferably is between 0.1 mg cm^{-2} and 15 mg cm^{-2} and even more preferably is between 0.5 mg cm^{-2} and 10 mg cm^{-2} , in the case that the penetrant is applied on said nasal or other mucosa.

20

In another advantageous aspect of the invention, a kit containing said formulation in an amount which enables the formulation to be applied at the selected dose per area as afore-mentioned is provided.

25

It then is preferred if the formulation is contained in a bottle or any other packaging vessel.

The kit preferably contains a device for administering the formulation.

According to another aspect of the present invention a patch is provided containing the formulation in an amount that yields the dose per area as mentioned
5 above. The patch or transdermal patch according to the present invention is intended for the application to barriers including the skin, mucosa or plants. The term "transdermal" should include these aforesaid barriers.

Preferably the patch comprises

- 10
- a non-occlusive backing liner;
 - an inner liner, wherein the backing liner and the inner liner define a reservoir, and /or a matrix layer.

It is preferred that said non-occlusive backing liner exhibits a mean vapor
15 transmission rate (MVTR) of more than 1000 g/m²day, preferably of more than 5.000 g/m²day and most preferably of more than 10.000 g/m²day. It is preferred that the non-occlusive backing liner has pores of smaller than 100 nm, preferably smaller than 70 nm, more preferably of smaller than 30 nm and most preferably as big as the inter-molecular distances of the backing material. In a further preferred
20 embodiment the non-occlusive backing liner comprises a polyurethane membrane, preferably a polyester track-etched porous membrane, more preferably a polycarbonate track-etched porous membrane and most preferably a polyethylene microporous membrane.

25 The inner liner and / or matrix layer according to the present invention establishes skin contact. The inner liner preferably prevents unwanted release of the formulation from the patch during storage and enables rapid skin wetting when contacted with the skin. According to the present invention it is further preferred that the inner liner comprises a homogeneous membrane, preferably a polyester

track-etched porous membrane or a polycarbonate track-etched porous membrane. Moreover, these inner liner membranes preferably have a pore density of up to 5%, preferably of up to 15%, more preferably of up to 25% and most preferably of more than 25% and/or a pore size in the range between 20 nm and 200 nm,
5 preferably between 50 nm and 140 nm and most preferably between 80 nm and 120 nm.

Further preferred inner liner materials comprise a hydrophobic mesh-membrane and/or a nonwoven fleece with mesh openings formed by hydrophobic threads. In
10 another preferred embodiment the inner liner is a microporous polyethylene membrane having average pore sizes in the range of between 50 nm to 3000 nm, preferably between 500 nm to 2000 nm and most preferably of about 1500 nm.

According to a further preferred embodiment of the present invention the patch
15 comprises a pressure sensitive adhesive layer, preferably an adhesive layer comprising polyacrylate, polyisobutylene, silicone, ethylene vinyl acetate copolymer, polyvinylpyrrolidone or polyethylene oxide hydrogel.

According to another preferred feature of the present invention the formulation
20 comprises penetrants having an average diameter of smaller than 150 nm, preferably of smaller than 100 nm. It is also preferred that the total dry weight of droplets in the formulation is at least 5 weight-% (w-%), preferably between 7.5 w-% and 30 w-%, and more preferably between 10 w-% and 20 w-%.

25 The patch according to the present invention preferably comprises a formulation, wherein the formulation up to maximally 200 mPas, more preferably up to 40 mPas, and most preferably up to 8 mPas.

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The area of the drug releasing membrane is between 0.5 cm^2 and 250 cm^2 , more preferably is between 1 cm^2 and 100 cm^2 , even more preferably is between 2 cm^2 and 50 cm^2 and most preferred is between 4 cm^2 and 25 cm^2 .

- 5 In an especially preferred embodiment it is preferred that the patch comprises one or more additional layers comprising desiccant containing layers, matrix layers, foam tape layers and/or protective layers.

- 10 The inventors found that it is advantageous to use backing liners having the capability to support the evaporation of the Transfersomes suspending medium. According to the present invention they preferably exhibit a mean vapor transmission rate (MVTR) of more than $1000 \text{ g/m}^2\text{day}$ or, better, more than $10\,000 \text{ g/m}^2\text{day}$. The solvent disappearance across such a barrier at sufficiently high rate helps to create and to maintain an activity gradient which drives the flux of
- 15 Transfersome®-aggregates across a barrier.

- Suited inventive backing liners are polyurethane membranes, such as CoTran 9701 (3M Medica, Borken Germany), Tegaderm (3M Medica, Borken Germany), Arcare 8311 (Adhesive Research, Limerick, Ireland), IV3000 (Smith and
- 20 Nephew). Even better suited are polyester track-etched porous membranes (10 nm pore size) (Osmonics, Minnetonka, USA) and polycarbonate track-etched porous membranes (10 nm pore size) (Osmonics, Minnetonka, USA). Most suited are the polyethylene microporous membranes such as Cotran 9711 (3M Medica, Borken Germany), 14P01A, 10P05A, 8P07A, E011 D (DSM Solutech, Heerlen, The
- 25 Netherlands). In classical TTS known in the art, the latter materials customary are used for rate controlling membranes.

Said backing liner need to be liquid-tight in order to prevent loss of active substance, which should be delivered e.g. transdermally. In order to ensure or

determine if the membrane is liquid-tight, the penetrability of Transfersomes® through the membranes is measured upon application of low hydrostatic pressures. The polyethylene membranes Cotran 9711 (3M Medica, Borken Germany) and 14P01A are liquid tight up to an applied pressure of 1 MPa. Further, all cited
5 polyurethane membranes are liquid tight.

Another important feature of the patch according to the present invention is the use of an inner liner membrane instead of conventional rate controlling membranes which enable rapid skin wetting with the Transfersome®-formulation,
10 while blocking the (unwanted) release of the formulation during storage or during the application of the device on the skin. Since the present invention specifically is directed to Transfersome®-containing patches, the term "rate controlling membrane" is misleading, since the rate of Transfersome® mediated transport is ideally controlled by the water activity in and on the biological barrier. Thus, the
15 term "inner liner" is used herein instead of "rate controlling membrane".

One inner liner membrane, which is suitable for the purpose of the present invention is a homogeneous membrane having a high pore density. The passage through the pores depends on the Laplace pressure / surface tension of lipid
20 suspension within the pores $P_{\min} = 2 \sigma \cos \theta / r$, where P_{\min} denotes the minimal pressure required to overcome the Laplace pressure, σ is the surface tension of the suspension-air interface (~ 30 mN/m), θ is the contact angle of the formulation on the membrane material and r is the pore radius (~ 100 nm). Accordingly, retention of the formulation in the pores requires $\cos \theta < 0$, which means that the membrane
25 needs to be hydrophobic. According to this possible theory a Laplace pressure of 0.6 MPa is needed to move the air-suspension interface through the pores, thus enabling the suspension to cross the barrier.

Well suited inner liner membrane materials according to the present invention are polyester track-etched porous membranes (100 nm pore size) (Infiltec, Speyer, Germany) and polycarbonate track-etched porous membranes (100 nm pore size) (Infiltec, Speyer, Germany).

5

Morover, it is intended by the inventors to use hydrophobic mesh-membranes e.g. Fluortex 09/70/22, Fluortex 09/85/27 (INFILTEC, Speyer) and nonwoven fleeces e.g. Parafil R20, Parafil RK 20, Parafil R 30 Natur, Parafil RK 30, Paratherm PR 220/18, Paratherm PR 220/20 (LTS, Andernach, Germany). These sieving materials are well suited to act as inner liner in inventive patches.

10

Said liners constitute mesh openings built up by the hydrophobic threads. They prevent the passage of Transfersomes® when the liner is not in contact with the skin. The high contact angle γ of the air/water or air/Transfersome®-suspension interface, with respect to the hydrophobic surface of the thread, ensures this. The mesh openings allow for the passage of the Transfersomes® through the liner when contacting the skin. This is caused by the energy gained by the wetting of a more hydrophilic or of a less hydrophobic surface (e.g. the skin) exceeding the surface energy needed for the complete wetting of the threads.

15

20

In more concrete terms, said "switching-effect" can be explained as follows: Let d be the distance between two threads from midpoint to midpoint. Let r be the radius of a thread:

25

$$2 \pi r z \gamma_{wt} \approx z d \gamma_{ws}$$

The surface tension of water on the skin is $\gamma_{ws} = 40 \text{ m N/m}$ according to "Transdermal and Drug Delivery Systems", Buffalo Grove, Interpharm Press, Ghosh, Pfister et al. 1997. The surface tension of water on the hydrophobic thread

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is $\gamma_{wt} = 70 \text{ m N/m}$. (The surface tension of a suspension on skin is again $\gamma_{ws} = 40 \text{ m N/m}$, the surface tension of the suspension on the hydrophobic thread is $\gamma_{wt} = 35 \text{ m N/m}$, due to the presence of a detergent monolayer). Rearranging the above formula yields

$$2 \pi r / d \approx \gamma_{ws} / \gamma_{wt}$$

for the case of the suspension ($\gamma_{ws} \sim \gamma_{wt}$). This suggests that the thread radius to mesh size ratio should preferably be in the range of about 0.3.

According to the present invention it is especially preferred to use microporous polyethylene membranes as inner liner. The term "microporous" for the purposes of the present invention means pore sizes of at least 20 nm, preferably in the range between 50 nm to 3000 nm. Examples are Solupor - E011 D (mean pore size 1500 nm), Solupor - 8P07A (mean pore size 700 nm) and Solupor - 10P05A (mean pore size 500 nm) (DSM Solutech, Heerlen, The Netherlands), which exhibit a high penetrability at small pressures thus allowing for Transfersomes to wet the skin upon contact.

For all types of the above mentioned inner liner membranes the surface tension, σ , and the contact angle, γ , are changed when contacted with the skin. There are various factors, which can cause said changes of the surface tension, σ , and the contact angle, γ . One factor may be an increase in humidity and capillary condensation of transcutaneously released water. Hydrophilic bridging due to interaction between corneocytes / hair follicles and the inner membrane may also contribute to rapid skin wetting. Finally, hydrophilisation of the pore core by contaminants, such as microscopic skin fragments, may alter the surface tension, σ , and the contact angle, γ . As a consequence, the minimal pressure P_{min} , which is

required to overcome the Laplace pressure, is reduced and the formulation can pass the inner liner and wet the skin surface.

5 Patches according to the present invention can be manufactured by different methods known in the art. On principle the lamination of the backing and the inner liner can be carried out by heat lamination or adhesive lamination or any other known lamination method.

10 In heat lamination processes the liners are adhered by melting at least one material at elevated temperatures and elevated pressures for short periods. The melt(s) merge and intercalate upon cooling and consolidation. The temperature and pressure is applied by metallic chops, either pulse heated, e.g. by microwave radiation, or continuously heated. Polyethylene and polyurethan membranes typically are heat laminated at temperatures of 120 - 200 °C, preferably of 140 –
15 160 °C and pressures of 1-6 bar, preferably of 3-4 bar. Good lamination properties are achieved for Transfersom® containing patches by applying a pressure of 4 bar for a period of about 0.1 - 5 seconds, preferably of about 1-2 seconds.

20 Adhesive lamination of the liners is achieved by a layer of pressure sensitive adhesive such as polyacrylate, polyisobutylene, silicone, ethylene vinyl acetate copolymer or polyvinylpyrrolidone and polyethylene oxide hydrogel adhesive (PVP/PEO). The adhesive liner is precut to the appropriate shape for example a concentric ring having a width of 1 cm. The backing and the inner liners are laminated to the ring and the patch is punched out of the web. Suitable films are
25 for example a pressure sensitive transfer film (Arcare 7396), a flexible plastic film coated on both sides with a medical grade pressure sensitive adhesive (Arcare 8570 clear polyester) or foam tapes (Polyolefin 3M 1777; 3M 1779; 3M 9751, polyvinyl chloride 3M 9772L) coated on both sides with pressure sensitive acrylate adhesive. The latter example mounts a reservoir of defined volume due to

the finite thickness of the foam tape, while the former two examples draw their Transfersome® containing volume by the elasticity and/or hidden area of the liners.

- 5 The filling of the one compartment reservoir type patch according to the present invention can be achieved by several methods known in the art.

One possible filling procedure is based on a two-step lamination process. In the first step, the main compartment is laminated while retaining a small orifice.

10 Through this port a tap or a tubing is induced and the Transfersome® formulation is injected into the preformed reservoir. After retraction of the tap or tubing the lamination of the port is finalized. Heat lamination as well as adhesive lamination can be used in said procedure. In the case of heat lamination the heat chop laminates a **C**-shaped ring. After the filling of the inner part of the **C**, the heat

15 chop is revolved by 45° and the heat lamination is repeated a second time now closing the open part of the **C**. In the case of adhesive lamination the release liner of the transfer tape is not removed completely thus allowing for the establishment of the filling port. After filling the rest of the release liner is removed and the port is sealed. Back-folding of the backing and/or the inner liner leads to the same

20 result: A collar-like port is formed, which is sealed by refolding the membranes after the filling process.

The form, fill and seal technique is well established and can also be used for the manufacture of the patches according to the present invention. In a first step the

25 film for the backing liner is moved over a trough of desired dimensions. The liner adopts this shape under vacuum and lines the trough. Then a tap fills the Transfersome® formulation into the trough. After the tap is retracted the inner liner membrane is applied onto the web. A concentric seal ring laminates both films either by heat lamination or adhesive lamination as described above.

5 In a further suitable process for making TTS the Transfersome® formulation is injected through a preinstalled tubing after the lamination process. The tubing is laterally inserted into the foam in the same way as a venous catheter is set for continuous injection. The tubing is connected to a Transfersome®-formulation filled syringe by a luer lock. The desired amount of formulation is injected into the reservoir and the tubing is removed and /or sealed if necessary.

10 In another important aspect of the present invention a patch is provided which is further characterised in that the patch comprises at least two compartments, which are separated from each other during storage. According to another aspect of the present invention a patch is provided containing the formulation in an amount that yields the dose per area as mentioned above, wherein the patch comprises several, more preferably less than 5, even more preferably 3, and most preferred 2 separate
15 inner compartments which are combined prior to or during the application of the formulation. Preferably at least one of the compartments is inside and / or outside the patch.

20 It is preferred that the formulation and / or the individual formulation components and/or the agent and / or the suspension / dispersion of penetrants without the agent are kept during the storage in several, preferably less than 5, more preferably in 3, and most preferred in 2 separate compartments of the patch which, in case, are combined prior to or during or after the application of the patch.

25 In another preferred embodiment the outer compartments comprise injection systems, preferably syringes, which are connected to the reservoir of the patch. It is preferred that the compartments are vertically stacked and /or are arranged side-by-side and / or one compartment is included in a second compartment, preferably without being fixed to the second compartment.

Preferably the compartments are inside the reservoir, which is defined by the backing liner and the inner liner. It is further preferred that the compartments are
5 separated from each other by a controllably openable barrier, preferably a membrane and /or by a plug and / or by a compartment-forming lamination.

According to the present invention combining and mixing of the ingredients of the compartments is achieved by direct mechanical action, such as pressing, rubbing,
10 kneading, twisting, tearing and /or indirectly by changing the temperature, osmotic pressure or electrical potential, thereby causing the removal or destroying of the separating barrier(s).

In a further preferred embodiment of the present invention the patch comprises
15 – an inventive non-occlusive backing liner
– a membrane defining a reservoir, which is divided in at least two compartments,
wherein the formulation directly contacts the skin when the formulation releases from the reservoir or compartments.

20 The inventive multicompartment reservoir-type patch comprises at least two separate compartments and a mixing compartment, wherein said mixing compartment may be an storage compartment containing one ingredient of the formulation or the formulation or may be an compartment, which is not filled
25 during the storage period.

According to the present invention the storage compartments containing the critical ingredients may be separated from the mixing compartment. The storage compartments are containing some, if not all, ingredients during the storage period

after preparation and prior to application. The mixing compartment serves to mix the separated ingredients after the storage period. After mixing the formulation is released onto the skin from the mixing compartment. The mixing compartment may have an adjustable area of skin contact to allow for area-dose control. This can be done by the merger of smaller subunits of mixing compartments.

The mixing compartment has to be in contact with the skin. This can be achieved either by

1. direct contact with the skin (no inner liner membrane) or
2. an inner liner membrane according to the present invention. Reference is made to the one-compartment patch described above. The identical inner liner membranes may be used for multicompartment TTS.

The number of storage compartments may be at least two and is depending on the respective longterm-incompatibilities of the ingredients.

The storage compartments may be part of the patch and may be made of the same material(s). The storage compartments may be - in the simplest form - two syringes containing the liquid ingredients, which are injected sequentially or simultaneously into the mixing chamber through one or more tubes. A twin-syringe of which the two pistons are connected facilitates simultaneous injection and constancy of the ingredients ratio. An additional tubing ideally with micro-arcs as used in HPLC sample preparation may cause turbulences of the merged liquid. A T-piece connector, ideally with turbulence chamber serves in the same manner. Thus, an optimal mixing of the components is achieved even at high viscosities and high lipid-concentrations.

The mixing compartment according to the present invention may be one separate compartment which is empty during storage but filled almost simultaneously,

when the patch is applied onto the skin, or it may be one of the existing storage compartments in which the other ingredients are being added from other storage compartments, or it may be created by the merger of two or more storage compartments.

5

The combining or mixing of the ingredients can be achieved by perforating or destroying the compartment-separating membranes. This can be done, for example, by pressing or kneading the patch such that the compartment-separating membranes rupture upon this mechanical stress, or by the external or internal activation of a sharp tool, such as a needle by perforating the compartment-separating membrane.

10

Another method combining or mixing of the ingredients is based on opening a tube-system between the compartments. Said opening can be achieved e.g. by pressing or kneading the patch such that plug or squid which close the tubing between the separated compartments during the storage-period is released from the tubing due to the applied pressure.

15

It is also possible according to present invention to combine and mix the ingredients by unsealing of a lamination, which forms the separated storage compartments. This can be done, for example, by applying a small but a steady-state pressure onto the filled storage chambers, but also by heat lamination or adhesive lamination. The lamination of the compartment-forming membranes unseals and the liquids squeeze through the self-formed channels into the mixing compartment.

20

25

The storage and mixing compartments may be stacked vertically or placed side-by-side. For example, three membranes can be laminated in a manner that half of the middle membrane is sealed to the lower (e.g. inner liner) membrane and the

other half is sealed to the upper membrane (backing liner). Upper and lower membranes are sealed at the edges on the very right, very left, forward-turned and backward-turned sides thus forming a two-compartment pouch. The middle membrane might be impermeable to liquids, but also easy to disrupt. Suitable materials for middle membranes might be e.g. thin polyurethanes. According to one possible embodiment the storage container for the Transfersomes®-formulation may be the left liquid-tight compartment, while the Transfersome®-release is performed from the right chamber through the inventive inner liner membrane when contacted to the skin. The right chamber may serve e.g. as a storage compartment for (lyophilized) drug(s). It is clear to someone skilled in the art that also combinations of the aforementioned embodiments, e.g. a combination of the vertical stacking and side-by-side alignment are suitable for the purposes of the present invention.

After the mixing process in the mixing compartment the emptied storage compartments are dispensable. They may be unplugged (in the case of external compartments, such as syringes) or clipped off. For example the tubes may be detached and the ports may be sealed with tape or squids or plugs. Open sealing may be re-laminated by applying pressure.

It another important aspect of the present invention, a method is provided of administering an agent to a mammalian body or a plant, by transporting said agent through a barrier, wherein the barrier is the intact skin, mucosa and/or cuticle of said mammalian body or a plant, said agent being associated to a penetrant capable of transporting said agent through the skin pores or through the passages in mucosa or cuticle, or capable of enabling agent permeation through skin pores after said penetrant has opened and/or entered said pores, comprising the steps of:

- preparing a formulation by suspending or dispersing said penetrants in a polar liquid in the form of fluid droplets surrounded by a membrane-likc coating of

one or several layers, said coating comprising at least two kinds or forms of amphiphilic substances with a tendency to aggregate, provided that

- said at least two substances differ by at least a factor of 10 in solubility in said polar liquid,
- 5 - and / or said substances when in the form of homo-aggregates (for the more soluble substance) or of hetero-aggregates (for any combination of both said substances) have a preferred average diameter smaller than the diameter of homo-aggregates containing merely the less soluble substance,
- and / or the more soluble substance tends to solubilise the droplet and the
10 content of such substance is to up to 99 mol-% of solubilising concentration or else corresponds to up to 99 mol-% of the saturating concentration in the unsolubilised droplet, whichever is higher,
- and / or the presence of the more soluble substance lowers the average elastic energy of the membrane-like coating to a value at least 5 times lower, more
15 preferably at least 10 times lower and most preferably more than 10 times lower, than the average elastic energy of red blood cells or of phospholipid bilayers with fluid aliphatic chains,
- said penetrants being able to transport agents through the pores of said barrier or being able to promote agent permeation through the pores of said skin after
20 penetrants have entered the pores,
- selecting a dose amount of said penetrants to be applied on a predetermined area of said barrier to control the flux of said penetrants across said barrier, and
- applying the selected dose amount of said formulation containing said penetrants onto said area of said porous barrier.

25 It then is preferred if the flux across said barrier is increased by enlarging the applied dose amount of said penetrants per area of barrier.

The pH of the formulation preferably is chosen to be between 3 and 10, more

preferably is between 4 and 9, and most preferably is between 5 and 8.

In this aspect of the invention, it then is preferred if the formulation comprises:

- 5 – at least one thickening agent in an amount to increase the formulation viscosity to maximally 5 Nm/s, more preferably up to 1 Nm/s, and most preferably up to 0.2 Nm/s, so that formulation spreading-over, and drug retention at the application area is enabled,
- 10 – and / or at least one antioxidant in an amount that reduces the increase of oxidation index to less than 100 % per 6 months, more preferably to less than 100 % per 12 months and most preferably to less than 50 % per 12 months
- 15 – and / or at least one microbicide in an amount that reduces the bacterial count of 1 million germs added per g of total mass of the formulation to less than 100 in the case of aerobic bacteria, to less than 10 in the case of entero-bacteria, and to less than 1 in the case of *Pseudomonas aeruginosa* or *Staphylococcus aureus*, after a period of 4 days.

Said at least one microbicide then preferably is added in an amount that reduces the bacterial count of 1 million germs added per g of total mass of the formulation to less than 100 in the case of aerobic bacteria, to less than 10 in the case of
20 entero-bacteria, and to less than 1 in the case of *Pseudomonas aeruginosa* or *Staphylococcus aureus*, after a period of 3 days, and more preferably after a period of 1 day.

Said thickening agent preferably is selected from the class of pharmaceutically
25 acceptable hydrophilic polymers, such as partially etherified cellulose derivatives, like carboxymethyl-, hydroxyethyl-, hydroxypropyl-, hydroxypropylmethyl- or methyl-cellulose; completely synthetic hydrophilic polymers such as polyacrylates, polymethacrylates, poly(hydroxyethyl)-, poly(hydroxypropyl)-, poly(hydroxypropylmethyl)methacrylates, polyacrylonitriles, methallyl-

- 5 sulphonates, polyethylenes, polyoxiethylenes, polyethylene glycols, polyethylene glycol-lactides, polyethylene glycol-diacrylates, polyvinylpyrrolidones, polyvinyl alcohols, poly(propylmethacrylamides), poly(propylene fumarate-co-ethylene glycols), poloxamers, polyaspartamides, (hydrazine cross-linked) hyaluronic acids, silicones; natural gums comprising alginates, carrageenans, guar-gums, gelatines, tragacanth, (amidated) pectins, xanthans, chitosan collagens, agaroses; mixtures and further derivatives or co-polymers thereof and / or other pharmaceutically, or at least biologically, acceptable polymers.
- 10 The concentration of said polymer then preferably is chosen to be in the range between 0.01 w- % and 10 w- %, more preferably in the range between 0.1 w- % and 5 w- %, even more preferably in the range between 0.25 w- % and 3.5 w- % and most preferably in the range between 0.5 w- % and 2 w- %.
- 15 According to the invention said anti-oxidant then preferably is selected from synthetic phenolic antioxidants, such as butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT) and di-tert-butylphenol (LY178002, LY256548, HWA-131, BF-389, CI-986, PD-127443, E-5119, BI-L-239XX, etc.), tertiary butylhydroquinone (TBHQ), propyl gallate (PG), 1-O-hexyl-2,3,5-
- 20 trimethylhydroquinone (HTHQ); aromatic amines (such as diphenylamine, p-alkylthio-o-anisidine, ethylenediamine derivatives, carbazol, tetrahydroindenoindol); phenols and phenolic acids (such as guaiacol, hydroquinone, vanillin, gallic acids and their esters, protocatechuic acid, quinic acid, syringic acid, ellagic acid , salicylic acid, nordihydroguaiaretic acid (NDGA),
- 25 eugenol); tocopherols (including tocopherols (alpha, beta, gamma, delta) and their derivatives, such as tocopheryl-acylate (e.g. -acetate, -laurate, myristate, -palmitate, -oleate, -linoleate, etc., or any other suitable tocopheryl-lipoate), tocopheryl-POE-succinate; trolox and corresponding amide- and thiocarboxamide analogues; ascorbic acid and its salts, isoascorbate, (2 or 3 or 6)-o-alkylascorbic

acids, ascorbyl esters (e.g. 6-o-lauroyl, myristoyl, palmitoyl-, oleoyl, or linoleoyl-L-ascorbic acid, etc.); non-steroidal anti-inflammatory agents (NSAIDs), such as indomethacin, diclofenac, mefenamic acid, flufenamic acid, phenylbutazone, oxyphenbutazone acetylsalicylic acid, naproxen, diflunisal, 5 ibuprofen, ketoprofen, piroxicam, penicillamine, penicillamine disulphide, primaquine, quinacrine, chloroquine, hydroxychloroquine, azathioprine, phenobarbital, acetaminophen); aminosalicic acids and derivatives; methotrexate, probucol, antiarrhythmics (e.g. amiodarone, aprindine, asocainol), ambroxol, tamoxifen, b-hydroxytamoxifen; calcium antagonists (such as 10 nifedipine, nisoldipine, nimodipine, nicardipine, nilvadipine), beta-receptor blockers (e.g. atenolol, propranolol, nebivolol); sodium bisulphite, sodium metabisulphite, thiourea; chelating agents, such as EDTA, GDTA, desferral; endogenous defence systems, such as transferrin, lactoferrin, ferritin, cearyluloplasmin, haptoglobin, haemopexin, albumin, glucose, ubiquinol-10; 15 enzymatic antioxidants, such as superoxide dismutase and metal complexes with a similar activity, including catalase, glutathione peroxidase, and less complex molecules, such as beta-carotene, bilirubin, uric acid; flavonoids (e.g. flavones, flavonols, flavonones, flavanonals, chalcones, anthocyanins), N-acetylcystein, mesna, glutathione, thiohistidine derivatives, triazoles; tannins, cinnamic acid, 20 hydroxycinnamic acids and their esters (e.g. coumaric acids and esters, caffeic acid and their esters, ferulic acid, (iso-) chlorogenic acid, sinapic acid); spice extracts (e.g. from clove, cinnamon, sage, rosemary, mace, oregano, allspice, nutmeg); carnosic acid, carnosol, carsolic acid; rosmarinic acid, rosmarinidiphenol, gentisic acid, ferulic acid; oat flour extracts, such as avenanthramide 1 and 2; 25 thioethers, dithioethers, sulfoxides, tetralkylthiuram disulphides; phytic acid, steroid derivatives (e.g. U74006F); tryptophan metabolites (e.g. 3-hydroxykynurenine, 3-hydroxyanthranilic acid), and organochalcogenides, or else is an oxidation suppressing enzyme.

It then is preferred if the concentration of BHA or BHT is between 0.001 and 2 w-%, more preferably is between 0.0025 and 0.2 w-%, and most preferably is between 0.005 and 0.02 w-%, of TBHQ and PG is between 0.001 and 2 w-%, more preferably is between 0.005 and 0.2 w-%, and most preferably is between 0.01 and 0.02 w-%, of tocopherols is between 0.005 and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most preferably is between 0.05 and 0.075 w-%, of ascorbic acid esters is between 0.001 and 5, more preferably is between 0.005 and 0.5, and most preferably is between 0.01 and 0.15 w-%, of ascorbic acid is between 0.001 and 5, more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01 and 0.1 w-%, of sodium bisulphite or sodium metabisulphite is between 0.001 and 5, more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01-0.15 w-%, of thiourea is between 0.0001 and 2 w-%, more preferably is between 0.0005 and 0.2, and most preferably is between 0.001-0.01 w-%, most typically 0.005 w-%, of cystein is between 0.01 and 5, more preferably is between 0.05 and 2 w-%, and most preferably is between 0.1 and 1.0 w-%, most typically 0.5 w-%, of monothioglycerol is between 0.01 and 5 w-%, more preferably is between 0.05 and 2 w-%, and most preferably is between 0.1-1.0 w-%, most typically 0.5 w-%, of NDGA is between 0.0005-2 w-%, more preferably is between 0.001-0.2 w-%, and most preferably is between 0.005-0.02 w-%, most typically 0.01 w-%, of glutathione is between 0.005 and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most preferably is between 0.05 and 0.2 w-%, most typically 0.1 w-%, of EDTA is between 0.001 and 5 w-%, even more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01 and 0.2 w-%, most typically between 0.05 and 0.975 w-%, of citric acid is between 0.001 and 5 w-%, even more preferably is between 0.005 and 3 w-%, and most preferably is between 0.01-0.2, most typically between 0.3 and 2 w-%.

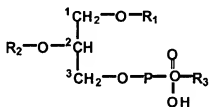
Preferrably said microbicide is then selected amongst short chain alcohols, such as ethyl and isopropyl alcohol, chlorbutanol, benzyl alcohol, chlorbenzyl alcohol, dichlorbenzylalcohol; hexachlorophene; phenolic compounds, such as cresol, 4-chloro-m-cresol, p-chloro-m-xilenol, dichlorophene, hexachlorophene, 5 povidon-iodine; parabens, especially alkyl-paraben, such as methyl-, ethyl-, propyl-, or butyl-paraben, benzyl-paraben; acids, such as sorbic acid, benzoic acid and its salts; quaternary ammonium compounds, such as alkonium salts, e.g. benzalkonium salts, especially the chlorides or bromides, cetrimonium salts, e.g. the bromide; phenoalkecinium salt, such as phenododecinium bromide, 10 cetylpyridinium chloride or other such salts; mercurium compounds, such as phenylmercuric acetate, borate, or nitrate, thiomersal; chlorhexidine or its gluconate; antibiotically active compounds of biological origin, or a mixture thereof.

15 It then is preferred that the bulk concentration of short chain alcohols in the case of ethyl, propyl, butyl or benzyl alcohol is up to 10 w-%, more preferably is up to 5 w-%, and most preferably is in the range between 0.5-3 w-%, and in the case of chlorbutanol is in the range between 0.3-0.6 w-%; bulk concentration of parabens, especially in the case of methyl paraben is in the range between 20 0.05-0.2 w-%, and in the case of propyl paraben is in the range between 0.002-0.02 w-%; bulk concentration of sorbic acid is in the range between 0.05-0.2 w-%, and in the case of benzoic acid is in the range between 0.1-0.5 w-%; bulk concentration of phenols, triclosan, is in the range between 0.1-0.3 w-%, and bulk concentration of chlorhexidine is in the range between 0.01-0.05 w-%.

25 It then is also preferred that the less soluble amongst the aggregating substances is a lipid or lipid-like material, especially a polar lipid, whereas the substance which is more soluble in the suspending liquid and which lowers the average elastic energy of the droplet is a surfactant or else has surfactant-like properties and / or is

a form of said lipid or lipid-like material which is comparably soluble as said surfactant or the surfactant-like material.

- 5 Preferably the lipid or lipid-like material is a lipid or a lipid from a biological source or a corresponding synthetic lipid or any of its modifications, said lipid preferably belonging to the class of pure phospholipids corresponding to the general formula



- 10 where R₁ and R₂ is an aliphatic chain, typically a C₁₀₋₂₀-acyl, or -alkyl or partly unsaturated fatty acid residue, in particular, an oleoyl-, palmitoeloyl-, elaidoyl-, linoleyl-, linolenyl-, linolenoyl-, arachidoyl-, vaccinyl-, lauroyl-, myristoyl-, palmitoyl-, or stearoyl chain; and where R₃ is hydrogen, 2-trimethylamino-1-ethyl,
- 15 2-amino-1-ethyl, C_{1,4}-alkyl, C_{1,5}-alkyl substituted with carboxy, C_{2,5}-alkyl substituted with hydroxy, C_{2,5}-alkyl substituted with carboxy and hydroxy, or C_{2,5}-alkyl substituted with carboxy and amino, inositol, sphingosine, or salts of said substances, said lipid comprising also glycerides, isoprenoid lipids, steroids,
- 20 sterines or sterols, of sulphur- or carbohydrate-containing lipids, or any other bilayer-forming lipids, in particular half-protonated fluid fatty acids, said lipid is selected from the group comprising phosphatidylcholines,
- phosphatidylethanolamines, phosphatidylglycerols, phosphatidylinositols, phosphatidic acids, phosphatidylserines, sphingomyelins or other sphingophospholipids, glycosphingolipids (including cerebrosides,

ceramidepolyhexosides, sulphatides, sphingoplasmalogens), gangliosides and other glycolipids or synthetic lipids, in particular with corresponding sphingosine derivatives, or any other glycolipids, whereby two similar or different chains can be ester-groups-linked to the backbone (as in diacyl and dialkenoyl compound) or
5 be attached to the backbone with ether bonds, as in dialkyl-lipids.

The surfactant or surfactant-like material preferably is a nonionic, a zwitterionic, an anionic or a cationic surfactant, especially a fatty-acid or -alcohol, an alkyl-tri/di/methyl-ammonium salt, an alkylsulphate salt, a monovalent salt of cholate,
10 deoxycholate, glycocholate, glycodeoxycholate, taurodeoxycholate, taurocholate, etc., an acyl- or alkanoyl-dimethyl- aminoxide, esp. a dodecyl- dimethyl- aminoxide, an alkyl- or alkanoyl-N-methylglucamide, N- alkyl-N,N-dimethylglycine, 3-(acyldimethylammonio)-alkanesulphonate, N-acyl-sulphobetaine, a polyethylene-glycol-octylphenyl ether, esp. a nonaethylene-glycol-octylphenyl ether, a polyethylene-acyl ether, esp. a nonaethylen-dodecyl
15 ether, a polyethylene-glycol-isoacyl ether, esp. a octaethylene-glycol-isotridecyl ether, polyethylene-acyl ether, esp. octaethylenedodecyl ether, polyethylene-glycol-sorbitane-acyl ester, such as polyethylenglykol-20-monolaurate (Tween 20) or polyethylenglykol-20-sorbitan-monooleate (Tween 80), a polyhydroxyethylene-
20 acyl ether, esp. polyhydroxyethylene- lauryl, -myristoyl, -cetylstearyl, or -oleoyl ether, as in polyhydroxyethylene-4 or 6 or 8 or 10 or 12, etc., -lauryl ether (as in Brij series), or in the corresponding ester, e.g. of polyhydroxyethylen-8-stearate (Myrj 45), -laurate or -oleate type, or in polyethoxylated castor oil 40, a sorbitane-monoalkylate (e.g. in Arlacel or Span), esp. sorbitane-monolaurate, an acyl- or
25 alkanoyl-N-methylglucamide, esp. in or decanoyl- or dodecanoyl-N-methylglucamide, an alkyl-sulphate (salt), e.g. in lauryl- or oleoyl-sulphate, sodium deoxycholate, sodium glycodeoxycholate, sodium oleate, sodium taurate, a fatty acid salt, such as sodium elaidate, sodium linoleate, sodium laurate, a lysophospholipid, such as n-octadecylene(=oleoyl)-glycerophosphatidic acid, -

phosphorylglycerol, or -phosphorylserine, n-acyl-, e.g. lauryl or oleoyl-glycero-phosphatidic acid, -phosphorylglycerol, or -phosphorylserine, n-tetradecyl-glycero-phosphatidic acid, -phosphorylglycerol, or -phosphorylserine, a
5 corresponding palmitoeloyl-, elaidoyl-, vaccenyl-lysophospholipid or a corresponding short-chain phospholipid, or else a surface-active polypeptide.

The average diameter of the penetrant preferably is between 30 nm and 500 nm, more preferably between 40 nm and 250 nm, even more preferably between 50 nm and 200 nm and particularly preferably between 60 nm and 150 nm.

10

The total dry weight of droplets in a formulation is then preferably chosen to range from 0.01 weight-% (w-%) to 40 w-% of total formulation mass, more preferably is between 0.1 w-% and 30 w-%, and most preferably is between 0,5 w-% and 20 w-%.

15

Preferably the total dry weight of droplets in a formulation is selected to increase the formulation viscosity to maximally 200 mPas, more preferably up to 40 mPas, and most preferably up to 8 mPas.

20

Preferably at least one edge-active substance or surfactant and/or at least one amphiphilic substance, and / or at least one hydrophilic fluid and the agent are mixed, if required separately, to form a solution, the resulting (partial) mixtures or solutions are then combined subsequently to induce, preferably by action of mechanical energy such as shaking, stirring, vibrations, homogenisation,
25 ultrasonication, shearing, freezing and thawing, or filtration using convenient driving pressure, the formation of penetrants that associate with and / or incorporate the agent

It also is preferred if said amphiphilic substances then are dissolved in volatile solvents, such as alcohols, especially ethanol, or in other pharmaceutically acceptable organic solvents, such as ethanol, 1- and 2-propanol, benzyl alcohol, propylene glycol, polyethylene glycol (molecular weight: 200-400 D) or glycerol, other pharmaceutically acceptable organic solvents, such as undercooled gas, especially supercritical CO₂, which are then removed, especially by evaporation or dilution, prior to making the final preparation.

The formation of said penetrants then preferably is induced by the addition of required substances into a fluid phase, evaporation from a reverse phase, by injection or dialysis, if necessary under the influence of mechanical stress, such as shaking, stirring, especially high velocity stirring, vibrating, homogenising, ultrasonication, shearing, freezing and thawing, or filtration using a convenient, especially low (1 MPa) or intermediate (up to 10 MPa), driving pressure.

It then is also preferred if the formation of said penetrants is induced by filtration, the filtering material having pores sizes between 0.01 µm and 0.8 µm, more preferably between 0.02 µm and 0.3 µm, and most preferably between 0.05 µm and 0.15 µm, whereby several filters may be used sequentially or in parallel.

Said agents and penetrants are made to associate, at least partly,

- after the formation of said penetrants, e.g. after injecting a solution of the drug in a pharmaceutically acceptable fluid, such as ethanol, 1- and 2-propanol, benzyl alcohol, propylene glycol, polyethylene glycol (molecular weight: 200-400 D) or glycerol into the suspending medium,
- simultaneously with penetrant formation, if required using the drug co-solution and, at least some, penetrant ingredients.

It then is preferred if said penetrants, with which the agent is associated, are prepared immediately before the application of the formulation, if convenient, from a suitable concentrate or a lyophilisate.

- 5 Accordingly the formulation is applied by spraying, smearing, rolling or sponging on the application area, in particular by using a metered sprayer, spender, roller or a sponge, or a non-occlusive patch, as appropriate.

- 10 It further is preferred if the barrier is skin or at least partly keratinised endothelium and / or nasal or any other mucosa.

- 15 The area dose of said penetrant then preferably is between 0.1 mg per square centimetre (mg cm^{-2}) and 40 mg cm^{-2} , more preferably is between 0.25 mg cm^{-2} and 30 mg cm^{-2} and even more preferably is between 0.5 mg cm^{-2} and 15 mg cm^{-2} , in the case that the penetrant is applied on said skin and / or said at least partly keratinised endothelium.

- 20 The area dose of said penetrant preferably is between 0.05 mg per square centimetre (mg cm^{-2}) and 20 mg cm^{-2} , more preferably is between 0.1 mg cm^{-2} and 15 mg cm^{-2} and even more preferably is between 0.5 mg cm^{-2} and 10 mg cm^{-2} , in the case that the penetrant is applied on said nasal or other mucosa.

- 25 The area dose of said penetrant preferably is between 0.0001 mg per square centimetre (mg cm^{-2}) and 0.1 mg cm^{-2} , more preferably is between 0.0005 mg cm^{-2} and 0.05 mg cm^{-2} and even more preferably is between 0.001 mg cm^{-2} and 0.01 mg cm^{-2} , in the case that the penetrant is applied on plant body, plant leaves or plant needles.

It is preferred if the method is used for generating an immune response on a human or other mammal by vaccinating said mammal.

5 It is preferred if the method is used for generating a therapeutic effect in a human or other mammal.

According to the present invention the above mentioned method is preferably used for the treatment of inflammatory disease, dermatosis, kidney or liver failure, adrenal insufficiency, aspiration syndrome, Behcet syndrome, bites and stings, 10 blood disorders, such as cold-haemagglutinin disease, haemolytic anemia, hypereosinophilia, hypoplastic anemia, macroglobulinaemia, trombocytopenic purpura, furthermore, for the management of bone disorders, cerebral oedema, Cogan's syndrome, congenital adrenal hyperplasia, connective tissue disorders, such as lichen, lupus erythematosus, polymyalgia rheumatica, polymyositis and 15 dermatomyositis, epilepsy, eye disorders, such as cataracts, Graves' ophthalmopathy, haemangioma, herpes infections, neuropathies, retinal vasculitis, scleritis, for some gastro-intestinal disorders, such as inflammatory bowel disease, nausea and oesophageal damage, for hypercalcaemia, infections, e.g. of the eye (as in infections mononucleosis), for Kawasaki disease, myasthenia gravis, various 20 pain syndromes, such as postherpetic neuralgia, for polyneuropathies, pancreatitis, in respiratory disorders, such as asthma, for the management of rheumatoid disease and osteoarthritis, rhinitis, sarcoidosis, skin diseases, such as alopecia, eczema, erythema multiforme, lichen, pemphigus and pemphigoid, psoriasis, pyoderma gangrenosum, urticaria, in case of thyroid and vascular disorders.

25 Without any limitation of the scope of the present invention as defined by the attached claims the invention shall now be described in more detail by referring to the following examples and figures only showing non-limiting embodiments of the present invention.

General experimental set-up and sample preparation

Test formulation. Highly adaptable aggregate droplets used within the framework of this work had the form of (oligo)bilayer vesicles. Typically, the test
5 formulation contained biocompatible (phospho)lipids, such as phosphatidylcholine, and (bio)surfactants, such as sodium cholate or polysorbate (Tween 80). Different phospholipid/detergent ratios have been chosen to maintain or select the highest possible aggregate deformability.

10 Manufacturing was done as described in previous applications of the applicant. In short, a solution of phosphatidylcholine (SPC; Natterman Phospholipids, Cologne, Germany) in chloroform was labelled with the tritiated SPC (Amersham, XXX) and mixed with sodium cholate (Merck, Darmstadt, Germany) to obtain a phospholipid/detergent ratio of 3.75/1 (mol/mol). The mixture was dispersed in
15 phosphate buffer (pH = 7.2) to yield a 10 w-% total lipid suspension.

Vesicles in the suspension were frozen and thawed three times. Subsequently, the formulation was passed under pressure through several micro-porous filters (first 200 nm; then 100 nm, and finally 50 nm or 80 nm; Poretics, CA). To check the
20 reproducibility of vesicle manufacturing, the average size of vesicles was measured with dynamic light scattering procedure and found to be in the range of 80 nm to 150 nm.

Test animals. Mice of NMRI strain were 8 to 12 weeks old at the time of
25 experimentation. They had free access to standard chow and water and were kept in suspension cages in groups of 4 to 6. Prior to test formulation administration, the application area on each animals back was shaved carefully. The test preparation was administered under general anaesthesia (0.3 mL per mouse of an isotonic NaCl solution containing 0.0071 % Rompun (Bayer, Leverkusen,

Germany) and 14.3 mg/mL Ketavet (Parke-Davis, Rochester, N.Y). The administration was done with a high precision pipette on the skin which was left non-occluded. Each animal was finally transferred into an individual cage where it was kept for a day. A different cage was used for each animal for at least 24 hrs.

5 4 animals were used per test group.

Test measurements. Blood samples were collected from tail end, after termination of experiment at least. In one set of experiments, the early blood sampling was done every 2 hrs. Organ samples included: liver, spleen, kidney,

10 and skin. The latter was also inspected superficially, by taking 10 strips (using a Tesa-Film).

Processing the organ samples was done according to standard procedures: for 3H-measurement, a small part of each organ and 100 μ L of the carcass lysate were

15 used to get the desired and quoted experimental data. These were analysed according to the standard procedures.

To determine total label recovery, the carcass of test animals was dissolved and discharged by addition of 50 mL perchloric acid

20

Recovery (% of applied activity) was determined and the recovered doses (% of applied activity per organ) as well as the total delivered amount [μ g lipid/g organ] were calculated.

25

Examples 1-5:Short term administration

5 Highly adaptable complex droplets (ultradeformable vesicles; Transfersomes)

87.4 mg phosphatidylcholine from soy bean (SPC)

12.6 mg sodium cholate (NaChol)

trace amount of ^3H -DPPC with specific activity: 750 $\mu\text{Ci}/500\mu\text{L}$

0.9 mL phosphate buffer, pH 7.3

10 Duration of experiment: 8 h .

Application area: 1 cm^2 on the upper dorsum. The various doses applied on the test area are given in the following table.

	Group 1	Group 2	Group 3	Group 4	Group 5
Applied volume [μL]	1.0	5.0	7.0	15.0	30.0
Appl. lipid amount [mg]	0.10	0.50	0.75	1.50	3.00
Applied activity [cpm]	108998	544991	817486	1634972	3269943

15 Results of test measurements are given in figures 1 to 6.

Examples 6-8:Longer term administration

20

Highly adaptable complex droplets (ultradeformable vesicles; Transfersomes)

87.4 mg phosphatidylcholine from soy bean (SPC)

12.6 mg sodium cholate (NaChol)

0.9 mL phosphate buffer, pH 7.3

25 trace amount of ^3H -DPPC with specific activity: 250 $\mu\text{Ci}/\text{mL}$

Duration of experiment: 24 h.

Application area: 1 cm squared; dose per area is given in the following table.

	Group 6	Group 7	Group 8
Applied volume [μ L]	10.0	50.0	100.0
Appl. lipid amount [mg]	1.00	5.00	10.00
Applied activity [cpm]	145599	727997	1E+06

5

To test the effect of changing administered dose per area over longer period of time, even greater suspension volumes were applied on upper back of test mice.

Resulting data are analysed and presented together with those from previous experimental series in figures 1 to 7.

10

Figure 1 shows the recovery of relative activity (penetrant amount) in different layers of the skin as a function of applied activity (dose).

Figure 2 shows the amount of carrier derived radioactivity (^3H -DPPC) in the blood as a function of time and epicutaneously administered penetrant quantity, expressed as percentage of applied dosage. As can be seen in this figure the relative amount of non-invasively administered lipid found in the blood reaches appreciable level after a clear lag-time of approximately 4 hours, but is nearly independent of the dose used.

20

Figure 3 indicates the relative accumulation of carrier derived radioactivity in various organs at two different time points after an increasing mass of ultradeformable carriers has been administered on the skin. It is apparent that whereas the relative amount of the carrier derived radioactivity decreases with the

25

applied dosage at both times of exploration, the phospholipid amount in the blood, viable skin and liver in parallel increases at $t = 8$ h, but remains nearly unchanged at $t = 24$ h.

5 Figure 4 shows the absolute penetrant distribution profile (in arbitrary units) in different layers of the skin as a function of applied activity (dose). Little dose dependence is seen in the horny layer for area doses between 0.5 mg cm^{-2} and up to 1.5 mg cm^{-2} , but greater penetrant amounts are deposited much more efficiently in the barrier. This is true 8 hours as well as 24 hours after the suspension
10 administration. Viable skin accumulates the penetrant derived material in a dose dependent fashion in entire investigated range.

Figure 5 shows the total amount of penetrant recovered in different tissues (skin, blood, liver) at different times after the administration of an increasing quantity of
15 ultra deformable penetrants on the skin grows with the applied dose per area. However, while at $t = 8$ h, an apparent saturation tendency is observed for doses greater than 1.5 mg cm^{-2} , at $t = 24$ h the dose dependence is linear.

Figure 6 shows the time dependence of penetrant derived radioactivity in the
20 blood as a function of epicutaneously administered suspension volume (lipid amount). As can be seen from this figure the temporal penetration characteristics are essentially independent of the applied dose: after a lag-time period of 4-6 hours, nearly steady state situation is observed.

25 Figure 7 shows the penetrant derived radioactivity in the blood as a function of epicutaneously administered dose measured 8 h or 24 h after the application. Linear extrapolation suggests that barrier starts to adapt itself to penetrant transport at approximately 0.75 mg cm^{-2} .

Non-occlusive one-compartment and multicompartment patches

Figure 8 shows the results obtained by measurement of the mean vapour transmission rate (MVTR) of five microporous polyethylene membranes, four polyurethan membranes and one polycarbonate track etched membrane.

Abbreviations used:

First akronym:

DSM	DSM Solutech, Heerlen, The Netherlands
3M	3M Medica, Borken, Germany
ARCare	Adhesives Research, Limerick, Ireland
SM	Smith and Nephew
Infiltec	Infiltec, Speyer, Germany

Second akronym:

PE	microporous polyethylen
PU	polyurethan
PCTE	polycarbonate track etched

The third akronym refers to the article number.

Figure 9 is a diagram showing the principle of the "switching-effect", which e.g. is observed in connection with the inventive hydrophobic mesh-membranes. A cross-section of two threads of a sieving material is given. In part 1 the threads are covered by a Transfersom®-formulation or lipid suspension without any contact to the skin, e.g. during storage. Contact with skin causes liquid bridges to the surface of the skin (part 2), which finally leads to complete skin wetting and release of Transfersomes® through the "sieve" (part 3).

Figure 10 shows the penetrability of three different microporous polyethylen membranes for Transfersomes®, namely Type-C; Solupor - E011 D, Solupor - 8P07A and Solupor - 10P05A (DSM Solutech, Heerlen, The Netherlands). They

exhibit a high penetrability at small pressures thus allowing for Transfersomes to wet the skin upon contact. Moreover, it can be taken from the figure, that no penetration of the Transfersomes® through the membranes is observed, when the pressure is 0.

5

Figure 11 shows a schematic diagram of a multicompartment patch having external compartments according to the present invention in form of twin syringe serving as storage compartments with mixing tubing or T-piece connector attached to the patch.

10

Figure 12 shows a schematic diagram of a multicompartment patch according to the present invention having vertically stacked compartments.

15

Figure 13 shows a schematic diagram of a multicompartment patch according to the present invention with a side-by-side alignment of compartments with vertically introduced septum.

20

Figure 14 shows a schematic diagram of a multicompartment patch according to the present invention having a side-by-side alignment of compartments with separating lamination.

25

An example for a patch, which is suited for application of a Transfersome®-formulation ($V = 0.6\text{mL}$) according to the present invention is given below. Said transdermal patch can be used as an one-compartment patch according to the present invention and also can be fitted with external compartments thereby producing a multicompartment patch according to the present invention.

- 61 -

Type	Material	Dimension
Backing liner	COTRAN 9701 / 3M 2 mil Polyurethan 70-0000-3993-6 SLP P261450106	Inner diameter 3.6 cm outer rectangle 4.5 cm *4.5 cm
Compartment	3M Foam tape 1779 polyolefin tape double layered # 70-0000-6467-8	
Inner liner	PCTE 100 nm Poretics; Cat 19410 LOT AE84AG11C024	
protective periphery	Leukoplast	
Injection tubing	Obturator Venflon 1.2 mm/18G L45 mm Art. No. 4253-1 LOT 931208	Preinstalled tubing; removed after TFS injection; port sealed with Leukoplast
Area of application	10 cm ²	
Application perimeter	3.6 cm	
Concentric seal width	> 0.8 cm	
Total area	20.25 cm ²	

Another example for a patch, which is suited for application of a Transfersome®-formulation according to the present invention is given below. Said patch has no inner liner membrane and is intended for direct application to the skin. Filling of

the mixing compartment (formed by the backing liner and the skin) can be done e.g. by external syringes connected to the mixing compartment.

Type	Material	Dimension
Backing liner	microporous Polyethylene 9711; 3M Medica #KG-90054	6 cm * 8.6 cm rectangle
Compartment	3M Foam tape 1779 polyolefin tape double layered # 70-0000-6467-8	outer rectangle 6 cm * 8.6 cm inner perimeter 4.4 cm * 7 cm
release cover I	from foam tape	
protective periphery	Leukoplast	
Injection tubing	Obturator Venflon 1.2 mm/18G L45 mm Art. No. 4253-1 LOT 931208	Preinstalled tubing; removed after TFS injection; port sealed with Leukoplast
Area of application	25 cm ²	
Application perimeter	4.4 cm * 7 cm	
Concentric seal width	> 0.8 cm	
Total area	51.6 cm ²	

C L A I M S

1. A method for controlling the flux of penetrants across an adaptable
5 semi-permeable porous barrier comprising the steps of:
- preparing a formulation by suspending or dispersing said penetrants in a polar
liquid in the form of fluid droplets surrounded by a membrane-like coating of
one or several layers, said coating comprising at least two kinds or forms of
amphiphilic substances with a tendency to aggregate, provided that
 - 10 – said at least two substances differ by at least a factor of 10 in solubility in said
polar liquid,
 - and / or said substances when in the form of homo-aggregates (for the more
soluble substance) or of hetero-aggregates (for any combination of both said
substances) have a preferred average diameter smaller than the diameter of
 - 15 homo-aggregates containing merely the less soluble substance,
 - and / or the more soluble substance tends to solubilise the droplet and the
content of such substance is to up to 99 mol-% of solubilising concentration or
else corresponds to up to 99 mol-% of the saturating concentration in the
unsolubilised droplet, whichever is higher;
 - 20 – and / or the presence of the more soluble substance lowers the average elastic
energy of the membrane-like coating to a value at least 5 times lower, more
preferably at least 10 times lower and most preferably more than 10 times
lower, than the average elastic energy of red blood cells or of phospholipid
bilayers with fluid aliphatic chains,
 - 25 – said penetrants being able to transport agents through the pores of said barrier
or to enable agent permeation through the pores of said barrier after penetrants
have entered the pores,
 - selecting a dose amount of said penetrants to be applied on a predetermined
area of said barrier to control the flux of said penetrants across said barrier, and

- applying the selected dose amount of said formulation containing said penetrants onto said area of said porous barrier.

2. The method according to claim 1,
5 **characterised in that** the flux across said barrier is increased by enlarging the applied dose per area of said penetrants.

3. The method according to claims 1 or 2,
10 **characterised in that** the pH of the formulation is between 3 and 10, more preferably between 4 and 9, and most preferably between 5 and 8.

4. The method according to any one of the preceding claims,
characterised in that the formulation comprises:
15 - at least one thickening agent in an amount that increases the formulation viscosity to maximally 5 Nm/s, more preferably up to 1 Nm/s, and most preferably up to 0.2 Nm/s, so that formulation spreading-over, and drug retention at the application area is enabled,
- and / or at least one antioxidant in an amount that reduces the increase of
20 oxidation index to less than 100 % per 6 months, more preferably to less than 100 % per 12 months and most preferably to less than 50 % per 12 months
- and / or at least one microbicide in an amount that reduces the bacterial count of 1 million germs added per g of total mass of the formulation to less than 100
in the case of aerobic bacteria, to less than 10 in the case of entero-bacteria, and to less than 1 in the case of *Pseudomonas aeruginosa* or *Staphylococcus aureus*,
25 after a period of 4 days.

5. The method according to claim 4,
characterised in that said at least one microbicide is added in an amount that reduces the bacterial count of 1 million germs added per g of total mass of the

formulation to less than 100 in the case of aerobic bacteria, to less than 10 in the case of entero-bacteria, and to less than 1 in the case of *Pseudomonas aeruginosa* or *Staphylococcus aureus*, after a period of 3 days, and more preferably after a period of 1 day.

5

6. The method according to claim 4,

characterised in that said thickening agent is selected from the class of pharmaceutically acceptable hydrophilic polymers, such as partially etherified cellulose derivatives, like carboxymethyl-, hydroxyethyl-, hydroxypropyl-,
10 hydroxypropylmethyl- or methyl-cellulose; completely synthetic hydrophilic polymers such as polyacrylates, polymethacrylates, poly(hydroxyethyl)-, poly(hydroxypropyl)-, poly(hydroxypropylmethyl)methacrylates, polyacrylonitriles, methallyl-sulphonates, polyethylenes, polyoxiethylenes, polyethylene glycols, polyethylene glycol-lactides, polyethylene glycol-diacrylates,
15 polyvinylpyrrolidones, polyvinyl alcohols, poly(propylmethacrylamides), poly(propylene fumarate-co-ethylene glycols), poloxamers, polyaspartamides, (hydrazine cross-linked) hyaluronic acids, silicones; natural gums comprising alginates, carrageenans, guar-gums, gelatines, tragacanth, (amidated) pectins, xanthans, chitosan collagens, agaroses; mixtures and further derivatives or
20 co-polymers thereof and / or other pharmaceutically, or at least biologically, acceptable polymers.

7. The method according to claim 6,

characterised in that the concentration of said polymer is in the range between
25 0.01 w- % and 10 w- %, more preferably in the range between 0.1 w- % and 5 w- %, even more preferably in the range between 0.25 w- % and 3.5 w- % and most preferably in the range between 0.5 w- % and 2 w- %.

8. The method according to claim 4,

characterised in that said anti-oxidant is selected from synthetic phenolic antioxidants, such as butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT) and di-tert-butylphenol (LY178002, LY256548, HWA-131, BF-389, CI-986, PD-127443, E-51119, BI-L-239XX, etc.), tertiary butylhydroquinone (TBHQ), propyl gallate (PG), 1-O-hexyl-2,3,5-trimethylhydroquinone (HTHQ); aromatic amines (such as diphenylamine, p-alkylthio-o-anisidine, ethylenediamine derivatives, carbazol, tetrahydroindenoindol); phenols and phenolic acids (such as guaiacol, hydroquinone, vanillin, gallic acids and their esters, protocatechuic acid, quinic acid, syringic acid, ellagic acid, salicylic acid, nordihydroguaiaretic acid (NDGA), eugenol); tocopherols (including tocopherols (alpha, beta, gamma, delta) and their derivatives, such as tocopheryl-acylate (e.g. -acetate, -laurate, myristate, -palmitate, -oleate, -linoleate, etc., or any other suitable tocopheryl-lipoate), tocopheryl-POE-succinate; trolox and corresponding amide- and thiocarboxamide analogues; ascorbic acid and its salts, isoascorbate, (2 or 3 or 6)-o-alkylascorbic acids, ascorbyl esters (e.g. 6-o-lauroyl, myristoyl, palmitoyl-, oleoyl, or linoleoyl-L-ascorbic acid, etc.); non-steroidal anti-inflammatory agents (NSAIDs), such as indomethacin, diclofenac, mefenamic acid, flufenamic acid, phenylbutazone, oxyphenbutazone acetylsalicylic acid, naproxen, diflunisal, ibuprofen, ketoprofen, piroxicam, penicillamine, penicillamine disulphide, primaquine, quinacrine, chloroquine, hydroxychloroquine, azathioprine, phenobarbital, acetaminophen); aminosalicyclic acids and derivatives; methotrexate, probucol, antiarrhythmics (e.g. amiodarone, aprindine, asocainol), amroxol, tamoxifen, b-hydroxytamoxifen; calcium antagonists (such as nifedipine, nisoldipine, nimodipine, nicardipine, nilvadipine), beta-receptor blockers (e.g. atenolol, propranolol, nebivolol); sodium bisulphite, sodium metabisulphite, thiourea; chelating agents, such as EDTA, GDTA, desferal; endogenous defence systems, such as transferrin, lactoferrin, ferritin, cearyluloplasmin, haptoglobin, haemopexin, albumin, glucose, ubiquinol-10; enzymatic antioxidants, such as superoxide dismutase and metal complexes with a

similar activity, including catalase, glutathione peroxidase, and less complex molecules, such as beta-carotene, bilirubin, uric acid; flavonoids (e.g. flavones, flavonols, flavonones, flavanols, chacones, anthocyanins), N-acetylcystein, mesna, glutathione, thiohistidine derivatives, triazoles; tannines, cinnamic acid, 5 hydroxycinnamic acids and their esters (e.g. coumaric acids and esters, caffeic acid and their esters, ferulic acid, (iso-) chlorogenic acid, sinapic acid); spice extracts (e.g. from clove, cinnamon, sage, rosemary, mace, oregano, allspice, nutmeg); carnosic acid, carnosol, carsolic acid; rosmarinic acid, rosmarindiphenol, gentisic acid, ferulic acid; oat flour extracts, such as avenanthramide 1 and 2; 10 thioethers, dithioethers, sulfoxides, tetraalkylthiuram disulphides; phytic acid, steroid derivatives (e.g. U74006F); tryptophan metabolites (e.g. 3-hydroxykynurenine, 3-hydroxyanthranilic acid), and organochalcogenides, or else is an oxidation suppressing enzyme.

15 9. The method according to claim 8, **characterised in that** the concentration of BHA or BHT is between 0.001 and 2 w-%, more preferably is between 0.0025 and 0.2 w-%, and most preferably is between 0.005 and 0.02 w-%, of TBHQ and PG is between 0.001 and 2 w-%, more preferably is between 0.005 and 0.2 w-%, and most preferably is between 20 0.01 and 0.02 w-%, of tocopherols is between 0.005 and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most preferably is between 0.05 and 0.075 w-%, of ascorbic acid esters is between 0.001 and 5, more preferably is between 0.005 and 0.5, and most preferably is between 0.01 and 0.15 w-%, of ascorbic acid is between 0.001 and 5, more preferably is between 0.005 and 0.5 w-%, and most 25 preferably is between 0.01 and 0.1 w-%, of sodium bisulphite or sodium metabisulphite is between 0.001 and 5, more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01-0.15 w-%, of thiourea is between 0.0001 and 2 w-%, more preferably is between 0.0005 and 0.2, and most preferably is between 0.001-0.01 w-%, most typically 0.005 w-%, of cystein is

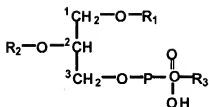
between 0.01 and 5, more preferably is between 0.05 and 2 w-%, and most preferably is between 0.1 and 1.0 w-%, most typically 0.5 w-%, of monothioglycerol is between 0.01 and 5 w-%, more preferably is between 0.05 and 2 w-%, and most preferably is between 0.1-1.0 w-%, most typically 0.5 w-%, of NDGA is between 0.0005-2 w-%, more preferably is between 0.001-0.2 w-%, and most preferably is between 0.005-0.02 w-%, most typically 0.01 w-%, of glutathione is between 0.005 and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most preferably is between 0.05 and 0.2 w-%, most typically 0.1 w-%, of EDTA is between 0.001 and 5 w-%, even more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01 and 0.2 w-%, most typically between 0.05 and 0.975 w-%, of citric acid is between 0.001 and 5 w-%, even more preferably is between 0.005 and 3 w-%, and most preferably is between 0.01-0.2, most typically between 0.3 and 2 w-%.

10. The method according claim 4,
characterised in that said microbicide is selected from short chain alcohols, such as ethyl and isopropyl alcohol, chlorbutanol, benzyl alcohol, chlorbenzyl alcohol, dichlorbenzylalcohol; hexachlorophene; phenolic compounds, such as cresol, 4-chloro-m-cresol, p-chloro-m-xlenol, dichlorophene, hexachlorophene, povidon-iodine; parabens, especially alkyl-paraben, such as methyl-, ethyl-, propyl-, or butyl-paraben, benzyl-paraben; acids, such as sorbic acid, benzoic acid and its salts; quaternary ammonium compounds, such as alkonium salts, e.g. benzalkonium salts, especially the chlorides or bromides, cetrimonium salts, e.g. the bromide; phenoalkecinium salt, such as phenododecinium bromide, cetylpyridinium chloride or other such salts; mercurium compounds, such as phenylmercuric acetate, borate, or nitrate, thiomersal; chlorhexidine or its gluconate; antibiotically active compounds of biological origin, or a mixture thereof.

11. The method according to claim 10,
characterised in that the bulk concentration of short chain alcohols in the case of ethyl, propyl, butyl or benzyl alcohol is up to 10 w-%, more preferably is up to 5 w-%, and most preferably is in the range between 0.5-3 w-%, and in the case of
5 chlorobutanol is in the range between 0.3-0.6 w-%; bulk concentration of parabens, especially in the case of methyl paraben is in the range between 0.05-0.2 w-%, and in the case of propyl paraben is in the range between 0.002-0.02 w-%; bulk concentration of sorbic acid is in the range between 0.05-0.2 w-%, and in the case of benzoic acid is in the range between 0.1-0.5 w-%;
10 bulk concentration of phenols, triclosan, is in the range between 0.1-0.3 w-%, and bulk concentration of chlorhexidine is in the range between 0.01-0.05 w-%.

12. The method according to any one of the preceding claims,
15 **characterised in that** the less soluble amongst the aggregating substances is a lipid or lipid-like material, especially a polar lipid, whereas the substance which is more soluble in the suspending liquid and which lowers the average elastic energy of the droplet is a surfactant or else has surfactant-like properties and / or is a form of said lipid or lipid-like material which is comparably as soluble as said
20 surfactant or the surfactant-like material.

13. The method according to claim 12,
characterised in that the lipid or lipid-like material is a lipid or a lipoid from a biological source or a corresponding synthetic lipid or any of its modifications,
25 said lipid preferably belonging to the class of pure phospholipids corresponding to the general formula



- where R₁ and R₂ is an aliphatic chain, typically a C₁₀₋₂₀-acyl, or -alkyl or partly unsaturated fatty acid residue, in particular, an oleoyl-, palmitoeloyl-, elaidoyl-, linoleyl-, linolenyl-, linolenoyl-, arachidoyl-, vacciny-, lauroyl-, myristoyl-, palmitoyl-, or stearoyl chain; and where R₃ is hydrogen, 2-trimethylamino-1-ethyl, 2-amino-1-ethyl, C₁₋₄-alkyl, C₁₋₅-alkyl substituted with carboxy, C₂₋₅-alkyl substituted with hydroxy, C₂₋₅-alkyl substituted with carboxy and hydroxy, or C₂₋₅-alkyl substituted with carboxy and amino, inositol, sphingosine, or salts of said substances, said lipid comprising also glycerides, isoprenoid lipids, steroids, sterines or sterols, of sulphur- or carbohydrate-containing lipids, or any other bilayer-forming lipids, in particular half-protonated fluid fatty acids, said lipid is selected from the group comprising phosphatidylcholines, phosphatidylethanolamines, phosphatidylglycerols, phosphatidylinositols, phosphatidic acids, phosphatidylserines, sphingomyelins or other sphingophospholipids, glycosphingolipids (including cerebrosides, ceramidepolyhexosides, sulphatides, sphingoplasmalogens), gangliosides and other glycolipids or synthetic lipids, in particular with corresponding sphingosine derivatives, or any other glycolipids, whereby two similar or different chains can be ester-groups-linked to the backbone (as in diacyl and dialkenoyl compound) or be attached to the backbone with ether bonds, as in dialkyl-lipids.

14. The method according to claim 12,

characterised in that the surfactant or surfactant-like material is a nonionic, a zwitterionic, an anionic or a cationic surfactant, especially a fatty-acid or -alcohol, an alkyl-tri/di/methyl-ammonium salt, an alkylsulphate salt, a monovalent salt of cholate, deoxycholate, glycocholate, glycodeoxycholate, taurodeoxycholate, taurocholate, etc., an acyl- or alkanoyl-dimethyl- aminoxide, esp. a dodecyl-dimethyl-aminoxide, an alkyl- or alkanoyl-N-methylglucamide, N- alkyl-N,N-dimethylglycine, 3-(acyldimethylammonio)-alkanesulphonate, N-acyl-sulphobetaine, a polyethylene-glycol-octylphenyl ether, esp. a nonaethylene-glycol-octylphenyl ether, a polyethylene-acyl ether, esp. a nonaethylen-dodecyl ether, a polyethylene-glycol-isoacyl ether, esp. a octaethylene-glycol-isotridecyl ether, polyethylene-acyl ether, esp. octaethylenedodecyl ether, polyethylene-glycol-sorbitane-acyl ester, such as polyethylen glykol-20-monolaurate (Tween 20) or polyethylen glykol-20-sorbitan-monooleate (Tween 80), a polyhydroxyethylene-acyl ether, esp. polyhydroxyethylene- lauryl, -myristoyl, -cetylstearyl, or -oleoyl ether, as in polyhydroxyethylene-4 or 6 or 8 or 10 or 12, etc., -lauryl ether (as in Brij series), or in the corresponding ester, e.g. of polyhydroxyethylen-8-stearate (Myrj 45), -laurate or -oleate type, or in polyethoxylated castor oil 40, a sorbitane-monoalkylate (e.g. in Arlacel or Span), esp. sorbitane-monolaurate, an acyl- or alkanoyl-N-methylglucamide, esp. in or decanoyl- or dodecanoyl-N-methylglucamide, an alkyl-sulphate (salt), e.g. in lauryl- or oleoyl-sulphate, sodium deoxycholate, sodium glycodeoxycholate, sodium oleate, sodium taurate, a fatty acid salt, such as sodium elaidate, sodium linoleate, sodium laurate, a lysophospholipid, such as n-octadecylene(=oleoyl)-glycerophosphatidic acid, -phosphorylglycerol, or -phosphorylserine, n-acyl-, e.g. lauryl or oleoyl-glycero-phosphatidic acid, -phosphorylglycerol, or -phosphorylserine, n-tetradecyl-glycero-phosphatidic acid, -phosphorylglycerol, or - phosphorylserine, a corresponding palmitoeloyl-, elaidoyl-, vaccenyl-lysophospholipid or a corresponding short-chain phospholipid, or else a surface-active polypeptide.

15. The method according to any of the preceding claims,
characterised in that the average diameter of the penetrant is between 30 nm and
500 nm, more preferably between 40 nm and 250 nm, even more preferably
5 between 50 nm and 200 nm and particularly preferably between 60 nm and
150 nm.

16. The method according to any one of the preceding claims,
characterised in that the total dry weight of droplets in a formulation is
10 0.01 weight-% (w-%) to 40 w-% of total formulation mass, more preferably
between 0.1 w-% and 30 w-%, and most preferably between 0,5 w-% and 20 w-%.

17. The method according to any one of the preceding claims,
characterised in that the total dry weight of droplets in a formulation is selected
15 to increase the formulation viscosity to maximally 200 mPas, more preferably up
to 40 mPas, and most preferably up to 8 mPas.

18. The method according to any one of the preceding claims,
characterised in that at least one amphiphilic substance and/or at least one edge-
20 active substance or surfactant, and / or at least one hydrophilic fluid and the agent
are mixed, if required separately, to form a solution, the resulting (partial)
mixtures or solutions are then combined subsequently to induce, preferably by
action of mechanical energy such as shaking, stirring, vibrations, homogenisation,
ultrasonication, shearing, freezing and thawing, or filtration using convenient
25 driving pressure, the formation of penetrants that associate with and / or
incorporate the agent

19. The method of claim 18,

5 **characterised in that** said amphiphilic substances are dissolved in volatile solvents, such as alcohols, especially ethanol, or in other pharmaceutically acceptable organic solvents, such as ethanol, 1- and 2-propanol, benzyl alcohol, propylene glycol, polyethylene glycol (molecular weight: 200-400 D) or glycerol, other pharmaceutically acceptable organic solvents, such as undercooled gas, especially supercritical CO₂, which are then removed, especially by evaporation or dilution, prior to making the final preparation.

10 20. The method according to any one of claims 18 or 19, **characterised in that** the formation of said penetrants is induced by the addition of required substances into a fluid phase, evaporation from a reverse phase, by injection or dialysis, if necessary under the influence of mechanical stress, such as shaking, stirring, in especially high velocity stirring, vibrating, homogenising, ultrasonication, shearing, freezing and thawing, or filtration using convenient, in 15 especially low (1 MPa) or intermediate (up to 10 MPa), driving pressure.

20 21. The method of claim 20, **characterised in that** the formation of said penetrants is induced by filtration, the filtering material having pores sizes between 0.01 µm and 0.8 µm, more preferably between 0.02 µm and 0.3 µm, and most preferably between 0.05 µm and 0.15 µm, whereby several filters may be used sequentially or in parallel.

25 22. The method according to any one of claims 18 to 21, **characterised in that** said agents and penetrants are made to associate, at least partly,
- after the formation of said penetrants, e.g. after injecting a solution of the drug in a pharmaceutically acceptable fluid, such as ethanol, 1- and 2-propanol, benzyl alcohol, propylene glycol, polyethylene glycol (molecular weight: 200-400 D) or glycerol into the suspending medium,

- simultaneously with penetrant formation , if required using the drug co-solution and, at least some, penetrant ingredients.

23. The method according to any one of the claims 18 to 22,
5 **characterised in that** said penetrants, with which the agent is associated, are prepared immediately before the application of the formulation, if convenient, from a suitable concentrate or a lyophilisate.

24. The method according to any one of the preceding claims,
10 **characterised in that** the formulation is applied by spraying, smearing, rolling or sponging on the application area, in particular by using a metering sprayer, spender, roller, sponge or a non-occlusive patch, as appropriate.

25. The method according to any one of the preceding claims,
15 **characterised in that** the barrier is a part of a mammalian body and / or a plant and preferably is skin and / or at least partly keratinised endothelium and / or nasal or any other mucosa.

26. The method according to claim 25,
20 **characterised in that**, the area dose of said penetrant is between 0.1 mg per square centimetre (mg cm^{-2}) and 40 mg cm^{-2} , more preferably is between 0.25 mg cm^{-2} and 30 mg cm^{-2} and even more preferably is between 0.5 mg cm^{-2} and 15 mg cm^{-2} , in case the penetrant is applied on said skin and / or said at least partly keratinised endothelium.

25
27. The method according to claim 25,
characterised in that the area dose of said penetrant is between 0.05 mg per square centimetre (mg cm^{-2}) and 20 mg cm^{-2} , more preferably is between 0.1 mg cm^{-2} and 15 mg cm^{-2} and even more preferably is between 0.5 mg cm^{-2}

and 10 mg cm⁻², in the case the penetrant is applied on said nasal or other mucosa.

28. The method according to claim 25,
5 **characterised in that** the area dose of said penetrant is between 0.0001 mg per square centimetre (mg cm⁻²) and 0.1 mg cm⁻², more preferably is between 0.0005 mg cm⁻² and 0.05 mg cm⁻² and even more preferably is between 0.001 mg cm⁻² and 0.01 mg cm⁻², in the case that the penetrant is applied on plant body, plant leaves or plant needles.

10

29. A kit containing said formulation in an amount which enables the formulation to be applied at the selected dose per area, according to any one of the preceding claims.

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30. The kit according to claim 29,
characterised in that the formulation is contained in a bottle or any other packaging vessel.

20

31. The kit according to claims 29 or 30,
characterised in that it contains a device for administering the formulation.

25

32. A patch, containing the formulation as in any one of claims 1 to 28 in an amount that yields the dose per area according to any one of the preceding claims.

33. The patch according to claim 32,
comprising:
— a non-occlusive backing liner;

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- an inner liner, wherein the backing liner and the inner liner define a reservoir;
and /or a matrix layer.

34. The patch according to claims 32 or 33,
5 **characterised in that** the non-occlusive backing liner exhibits a mean vapor transmission rate (MVTR) of more than 1000 g/m²day, preferably of more than 5.000 g/m²day and most preferably of more than 10.000 g/m²day.

35. The patch according to claims 32 or 34,
10 **characterised in that** the non-occlusive backing liner has pores of smaller than 100 nm, preferably smaller than 70 nm and most preferably of smaller than 30 nm.

36. The patch according to any one of claims 32 to 35,
15 **characterised in that** the non-occlusive backing liner comprises a polyurethane membrane, preferably a polyester track-etched porous membrane, more preferably a polycarbonate track-etched porous membrane and most preferably a polyethylene microporous membrane.

37. The patch according to any one of claims 32 to 36,
20 **characterised in that** the inner liner prevents unwanted release of the formulation from the patch during storage and enables rapid skin wetting when contacted with the skin.

38. The patch according to any one of claims 32 to 37,
25 **characterised in that** the inner liner comprises a homogeneous membrane, preferably a polyester track-etched porous membrane or a polycarbonate track-etched porous membrane.

39. The patch according to claim 38,

characterised in that the membranes have a pore density of up to 5%, preferably of up to 15%, more preferably of up to 25% and most preferably of more than 25% and/or a pore size in the range between 20 nm and 200 nm, preferably between 50 nm and 140 nm and most preferably between 80 nm and 120 nm.

5

40. The patch according to any one of claims 32 to 39,
characterised in that the inner liner comprises a hydrophobic mesh-membrane and/or a nonwoven fleece with mesh openings formed by hydrophobic threads.

10

41. The patch according to any one of claims 32 to 40,
characterised in that the inner liner comprises a microporous polyethylene membrane having average pore sizes in the range of between 50 nm to 3000 nm, preferably between 500 nm to 2000 nm and most preferably of about 1500 nm.

15

42. The patch according to any one of claims 32 to 41,
characterised in that the patch comprises a pressure sensitive adhesive layer, preferably an adhesive layer comprising polyacrylate, polyisobutylene, silicone, ethylene vinyl acetate copolymer, polyvinylpyrrolidone or polyethylene oxide hydrogel.

20

43. The patch according to any one of claims 32 to 42,
characterised in that the formulation viscosity is up to maximally 200 mPas, more preferably up to 40 mPas, and most preferably up to 8 mPas.

25

44. The patch according to any one of claims 32 to 43,
characterised in that the patch comprises one or more additional layers comprising desiccant containing layers, matrix layers, foam tape layers and/or protective layers.

45. The patch according to claim 32 to 44,
characterised in that the patch comprises at least two compartments, which are separated from each other during storage.

5 46. The patch according to claim 32 to 45,
characterised in that at least one of the compartments is inside and / or outside the patch.

10 47. The patch according to claim 32 to 46,
characterised in that the formulation and / or the individual formulation components and/or the agent and / or the suspension / dispersion of penetrants without the agent are kept during the storage in several, preferably less than 5, more preferably in 3, and most preferred in 2 separate compartments of the patch which, in case, are combined prior to or during or after the application of the
15 patch.

 48. The patch according to claim 32 to 47,
characterised in that the outer compartment(s) comprise(s) injection systems, which are connected to the reservoir.

20 49. The patch according to claim 32 to 47,
characterised in that the compartments are inside the reservoir, which is defined by the backing liner and the inner liner.

25 50. The patch according to claim 32 to 47,
characterised in that the compartments are vertically stacked and /or are arranged side-by-side and / or one compartment is included in a second compartment, preferably without being fixed to the second compartment.

51. The patch according to claim 49 or 50,
characterised in that the compartments are separated from each other by a controllably openable barrier, preferably a membrane and /or by a plug and / or by a compartment-forming lamination.

5

52. The patch according to claim 45 to 51,
characterised in that combining and mixing of the ingredients of the compartments is achieved by direct mechanical action, such as pressing, rubbing, kneading, twisting, tearing and /or indirectly by changing the temperature, osmotic pressure or electrical potential.

10

53. The patch according to claim 32,
comprising:
– a non-occlusive backing liner as in any of claims 34 to 37
– a membrane defining a reservoir, which is divided in at least two compartments,
characterised in that the formulation directly contacts the skin when the formulation releases from the reservoir.

15

54. A method of administering an agent to a mammalian body or a plant, by transporting said agent through a barrier, wherein the barrier is the intact skin, mucosa and/or cuticle of said mammalian body or a plant, said agent being associated to a penetrant capable of transporting said agent through the skin pores or through the passages in mucosa or cuticle, or capable of enabling agent permeation through skin pores after said penetrant has opened and/or entered said pores, comprising the steps of:
– preparing a formulation by suspending or dispersing said penetrants in a polar liquid in the form of fluid droplets surrounded by a membrane-like coating of

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- one or several layers, said coating comprising at least two kinds or forms of amphiphilic substances with a tendency to aggregate, provided that
- said at least two substances differ by at least a factor of 10 in solubility in said polar liquid,
 - 5 - and / or said substances when in the form of homo-aggregates (for the more soluble substance) or of hetero-aggregates (for any combination of both said substances) have a preferred average diameter smaller than the diameter of homo-aggregates containing merely the less soluble substance,
 - 10 - and / or the more soluble substance tends to solubilise the droplet and the content of such substance is to up to 99 mol-% of solubilising concentration or else corresponds to up to 99 mol-% of the saturating concentration in the unsolubilised droplet, whichever is higher,
 - 15 - and / or the presence of the more soluble substance lowers the average elastic energy of the membrane-like coating to a value at least 5 times lower, more preferably at least 10 times lower and most preferably more than 10 times lower, than the average elastic energy of red blood cells or of phospholipid bilayers with fluid aliphatic chains,
 - 20 - said penetrants being able to transport agents through the pores of said barrier or being able to promote agent permeation through the pores of said skin after penetrants have entered the pores,
 - 25 - selecting a dose amount of said penetrants to be applied on a predetermined area of said barrier to control the flux of said penetrants across said barrier, and
 - applying the selected dose amount of said formulation containing said penetrants onto said area of said porous barrier.

55. The method according to claim 54,
characterised in that the flux of penetrants across said barrier is increased by enlarging the applied dose per area of said penetrants.

56. The method according to claims 54 or 45,
characterised in that the pH of the formulation is between 3 and 10, more preferably between 4 and 9, and most preferably between 5 and 8.

5 57. The method according to claims 54 to 56,
characterised in that the formulation comprises:

- at least one thickening agent in an amount that increases the formulation viscosity to maximally 5 Nm/s, more preferably up to 1 Nm/s, and most preferably up to 0.2 Nm/s, so that formulation spreading-over, and drug
- 10 retention at the application area is enabled,
- and / or at least one antioxidant in an amount that reduces the increase of oxidation index to less than 100 % per 6 months, more preferably to less than 100 % per 12 months and most preferably to less than 50 % per 12 months
- 15 – and / or at least one microbicide in an amount that reduces the bacterial count of 1 million germs added per g of total mass of the formulation to less than 100 in the case of aerobic bacteria, to less than 10 in the case of entero-bacteria, and to less than 1 in the case of *Pseudomonas aeruginosa* or *Staphylococcus aureus*, after a period of 4 days.

20 58. Method according to claim 54,
characterised in that said at least one microbicide is added in an amount that reduces the bacterial count of 1 million germs added per g of total mass of the formulation to less than 100 in the case of aerobic bacteria, to less than 10 in the case of entero-bacteria, and to less than 1 in the case of *Pseudomonas aeruginosa* or *Staphylococcus aureus*, after a period of 3 days, and more preferably after a

25 period of 1 day.

59. The method according to claim 54,

characterised in that said thickening agent is selected from the class of pharmaceutically acceptable hydrophilic polymers, such as partially etherified cellulose derivatives, like carboxymethyl-, hydroxyethyl-, hydroxypropyl-, hydroxypropylmethyl- or methyl-cellulose; completely synthetic hydrophilic polymers such as polyacrylates, polymethacrylates, poly(hydroxyethyl)-, poly(hydroxypropyl)-, poly(hydroxypropylmethyl)methacrylates, polyacrylonitriles, methallyl-sulphonates, polyethylenes, polyoxiethylenes, polyethylene glycols, polyethylene glycol-lactides, polyethylene glycol-diacrylates, polyvinylpyrrolidones, polyvinyl alcohols, poly(propylmethacrylamides), poly(propylene fumarate-co-ethylene glycols), poloxamers, polyaspartamides, (hydrazine cross-linked) hyaluronic acids, silicones; natural gums comprising alginates, carrageenans, guar-gums, gelatines, tragacanth, (amidated) pectins, xanthans, chitosan collagens, agaroses; mixtures and further derivatives or co-polymers thereof and / or other pharmaceutically, or at least biologically, acceptable polymers.

60. The method according to claim 59, **characterised in that** the concentration of said polymer is in the range between 0.01 w- % and 10 w- %, more preferably in the range between 0.1 w- % and 5 w- %, even more preferably in the range between 0.25 w- % and 3.5 w- % and most preferably in the range between 0.5 w- % and 2 w- %.

61. The method according to claim 54, **characterised in that** said anti-oxidant is selected from synthetic phenolic antioxidants, such as butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT) and di-tert-butylphenol (LY178002, LY256548, HWA-131, BF-389, CI-986, PD-127443, E-5119, BI-L-239XX, etc.), tertiary butylhydroquinone (TBHQ), propyl gallate (PG), 1-O-hexyl-2,3,5-trimethylhydroquinone (HHTQ); aromatic amines (such as diphenylamine, p-alkylthio-o-anisidine, ethylenediamine

derivatives, carbazol, tetrahydroindenoindol); phenols and phenolic acids (such as guaiacol, hydroquinone, vanillin, gallic acids and their esters, protocatechuic acid, quinic acid, syringic acid, ellagic acid, salicylic acid, nordihydroguaiaretic acid (NDGA), eugenol); tocopherols (including tocopherols (alpha, beta, gamma, delta) and their derivatives, such as tocopheryl-acylate (e.g. -acetate, -laurate, myristate, -palmitate, -oleate, -linoleate, etc., or any other suitable tocopheryl-lipoate), tocopheryl-POE-succinate; trolox and corresponding amide- and thiocarboxamide analogues; ascorbic acid and its salts, isoascorbate, (2 or 3 or 6)-o-alkylascorbic acids, ascorbyl esters (e.g. 6-o-lauroyl, myristoyl, palmitoyl-, oleoyl, or linoleoyl-L-ascorbic acid, etc.); non-steroidal anti-inflammatory agents (NSAIDs), such as indomethacin, diclofenac, mefenamic acid, flufenamic acid, phenylbutazone, oxyphenbutazone acetylsalicylic acid, naproxen, diflunisal, ibuprofen, ketoprofen, piroxicam, penicillamine, penicillamine disulphide, primaquine, quinacrine, chloroquine, hydroxychloroquine, azathioprine, phenobarbital, acetaminophen); aminosalicic acids and derivatives; methotrexate, probucol, antiarrhythmics (e.g. amiodarone, aprindine, asocainol), ambroxol, tamoxifen, b-hydroxytamoxifen; calcium antagonists (such as nifedipine, nisoldipine, nimodipine, nicardipine, nilvadipine), beta-receptor blockers (e.g. atenolol, propranolol, nebivolol); sodium bisulphite, sodium metabisulphite, thiourea; chelating agents, such as EDTA, GDTA, desferral; endogenous defence systems, such as transferrin, lactoferrin, ferritin, ceruloplasmin, haptoglobin, haemopexin, albumin, glucose, ubiquinol-10; enzymatic antioxidants, such as superoxide dismutase and metal complexes with a similar activity, including catalase, glutathione peroxidase, and less complex molecules, such as beta-carotene, bilirubin, uric acid; flavonoids (e.g. flavones, flavonols, flavonones, flavanonals, chalcones, anthocyanins), N-acetylcystein, mesna, glutathione, thiohistidine derivatives, triazoles; tannines, cinnamic acid, hydroxycinnamic acids and their esters (e.g. coumaric acids and esters, caffeic acid and their esters, ferulic acid, (iso-) chlorogenic acid, sinapic acid); spice

extracts (e.g. from clove, cinnamon, sage, rosemary, mace, oregano, allspice, nutmeg); carnosic acid, carnosol, carsolic acid; rosmarinic acid, rosmarindiphenol, gentisic acid, ferulic acid; oat flour extracts, such as avenanthramide 1 and 2; thioethers, dithioethers, sulfoxides, tetralkylthiuram disulphides; phytic acid, steroid derivatives (e.g. U74006F); tryptophan metabolites (e.g. 3-hydroxykynurenine, 3-hydroxyanthranilic acid), and organochalcogenides, or else is an oxidation suppressing enzyme.

62. The method according to claim 54,
10 **characterised in that** the concentration of BHA or BHT is between 0.001 and 2 w-%, more preferably is between 0.0025 and 0.2 w-%, and most preferably is between 0.005 and 0.02 w-%, of TBHQ and PG is between 0.001 and 2 w-%, more preferably is between 0.005 and 0.2 w-%, and most preferably is between 0.01 and 0.02 w-%, of tocopherols is between 0.005 and 5 w-%, more preferably
15 is between 0.01 and 0.5 w-%, and most preferably is between 0.05 and 0.075 w-%, of ascorbic acid esters is between 0.001 and 5, more preferably is between 0.005 and 0.5, and most preferably is between 0.01 and 0.15 w-%, of ascorbic acid is between 0.001 and 5, more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01 and 0.1 w-%, of sodium bisulphite or sodium
20 metabisulphite is between 0.001 and 5, more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01-0.15 w-%, of thiourea is between 0.0001 and 2 w-%, more preferably is between 0.0005 and 0.2, and most preferably is between 0.001-0.01 w-%, most typically 0.005 w-%, of cystein is between 0.01 and 5, more preferably is between 0.05 and 2 w-%, and most
25 preferably is between 0.1 and 1.0 w-%, most typically 0.5 w-%, of monothioglycerol is between 0.01 and 5 w-%, more preferably is between 0.05 and 2 w-%, and most preferably is between 0.1-1.0 w-%, most typically 0.5 w-%, of NDGA is between 0.0005-2 w-%, more preferably is between 0.001-0.2 w-%, and most preferably is between 0.005-0.02 w-%, most typically 0.01 w-%, of

glutathione is between 0.005 and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most preferably is between 0.05 and 0.2 w-%, most typically 0.1 w-%, of EDTA is between 0.001 and 5 w-%, even more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01 and 0.2 w-%, most
5 typically between 0.05 and 0.975 w-%, of citric acid is between 0.001 and 5 w-%, even more preferably is between 0.005 and 3 w-%, and most preferably is between 0.01-0.2, most typically between 0.3 and 2 w-%.

63. The method according claim 54,
10 **characterised in that** said microbicide is selected amongst short chain alcohols, such as ethyl and isopropyl alcohol, chlorbutanol, benzyl alcohol, chlorbenzyl alcohol, dichlorbenzylalcohol; hexachlorophene; phenolic compounds, such as cresol, 4-chloro-m-cresol, p-chloro-m-xlenol, dichlorophene, hexachlorophene, povidon-iodine; parabens, especially alkyl-paraben, such as methyl-, ethyl-,
15 propyl-, or butyl-paraben, benzyl-paraben; acids, such as sorbic acid, benzoic acid and its salts; quaternary ammonium compounds, such as alkonium salts, e.g. benzalkonium salts, especially the chlorides or bromides, cetrimonium salts, e.g. the bromide; phenoalkecinium salt, such as phenododecinium bromide, cetylpyridinium chloride or other such salts; mercurium compounds, such as
20 phenylmercuric acetate, borate, or nitrate, thiomersal; chlorhexidine or its gluconate; antibiotically active compounds of biological origin, or a mixture thereof.

64. The method according claim 63,
25 **characterised in that** the bulk concentration of short chain alcohols in the case of ethyl, propyl, butyl or benzyl alcohol is up to 10 w-%, more preferably is up to 5 w-%, and most preferably is in the range between 0.5-3 w-%, and in the case of chlorbutanol is in the range between 0.3-0.6 w-%; bulk concentration of parabens, especially in the case of methyl paraben is in the range between

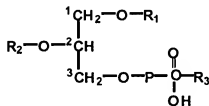
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0.05-0.2 w-%, and in the case of propyl paraben is in the range between
 0.002-0.02 w-%; bulk concentration of sorbic acid is in the range between 0
 .05-0.2 w-%, and in the case of benzoic acid is in the range between 0.1-0.5 w-%;
 bulk concentration of phenols, triclosan, is in the range between 0.1-0.3 w-%, and
 5 bulk concentration of chlorhexidine is in the range between 0.01-0.05 w-%.

65. The method according to claims 54 to 64,
characterised in that the less soluble amongst the aggregating substances is a
 lipid or lipid-like material, especially a polar lipid, whereas the substance which is
 10 more soluble in the suspending liquid and which lowers the average elastic energy
 of the droplet is a surfactant or else has surfactant-like properties and / or is a form
 of said lipid or lipid-like material which is comparably soluble as said surfactant
 or the surfactant-like material.

66. The method according to claim 65,
characterised in that the lipid or lipid-like material is a lipid or a lipoid from a
 biological source or a corresponding synthetic lipid or any of its modifications,
 said lipid preferably belonging to the class of pure phospholipids corresponding to
 the general formula

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where R₁ and R₂ is an aliphatic chain, typically a C₁₀₋₂₀-acyl, or -alkyl or partly
 unsaturated fatty acid residue, in particular, an oleoyl-, palmitoeloyl-, elaidoyl-,

linoleyl-, linolenyl-, linolenoyl-, arachidoyl-, vaccinyl-, lauroyl-, myristoyl-, palmitoyl-, or stearoyl chain; and where R₃ is hydrogen, 2-trimethylamino-1-ethyl, 2-amino-1-ethyl, C_{1,4}-alkyl, C_{1,5}-alkyl substituted with carboxy, C_{2,5}-alkyl substituted with hydroxy, C_{2,5}-alkyl substituted with carboxy and hydroxy, or C_{2,5}-alkyl substituted with carboxy and amino, inositol, sphingosine, or salts of said substances, said lipid comprising also glycerides, isoprenoid lipids, steroids, sterines or sterols, of sulphur- or carbohydrate-containing lipids, or any other bilayer-forming lipids, in particular half-protonated fluid fatty acids, said lipid is selected from the group comprising phosphatidylcholines, phosphatidylethanolamines, phosphatidylglycerols, phosphatidylinositols, phosphatidic acids, phosphatidylserines, sphingomyelins or other sphingophospholipids, glycosphingolipids (including cerebroside, ceramidepolyhexosides, sulphatides, sphingoplasmalogens), gangliosides and other glycolipids or synthetic lipids, in particular with corresponding sphingosine derivatives, or any other glycolipids, whereby two similar or different chains can be ester-groups-linked to the backbone (as in diacyl and dialkenoyl compound) or be attached to the backbone with ether bonds, as in dialkyl-lipids.

67. The method according to claim 65, **characterised in that** the surfactant or surfactant-like material preferably is a nonionic, a zwitterionic, an anionic or a cationic surfactant, especially a fatty-acid or -alcohol, an alkyl-tri/di/methyl-ammonium salt, an alkylsulphate salt, a monovalent salt of cholate, deoxycholate, glycocholate, glycodeoxycholate, taurodeoxycholate, taurocholate, etc., an acyl- or alkanoyl-dimethyl-aminoxide, esp. a dodecyl- dimethyl-aminoxide, an alkyl- or alkanoyl-N-methylglucamide, N-alkyl-N,N- dimethylglycine, 3-(acyldimethylammonio)-alkanesulphonate, N-acyl-sulphobetaine, a polyethylene-glycol-octylphenyl ether, esp. a nonaethylene-glycol-octylphenyl ether, a polyethylene-acyl ether, esp. a nonaethylen-dodecyl ether, a polyethylene-glycol-isoacyl ether, esp. a octaethylene-glycol-isotridecyl

ether, polyethylene-acyl ether, esp. octaethylenedodecyl ether, polyethylene-glycol-sorbitane-acyl ester, such as polyethyleneglykol-20-monolaurate (Tween 20) or polyethyleneglykol-20-sorbitan-monooleate (Tween 80), a polyhydroxyethylene-acyl ether, esp. polyhydroxyethylene- lauryl, -myristoyl, -cetylstearyl, or -oleoyl
5 ether, as in polyhydroxyethylene-4 or 6 or 8 or 10 or 12, etc., -lauryl ether (as in Brij series), or in the corresponding ester, e.g. of polyhydroxyethylen-8-stearate (Myrj 45), -laurate or -oleate type, or in polyethoxylated castor oil 40, a sorbitane-monoalkylate (e.g. in Arlacel or Span), esp. sorbitane-monolaurate, an acyl- or
10 alkanoyl-N-methylglucamide, esp. in or decanoyl- or dodecanoyl-N-methylglucamide, an alkyl-sulphate (salt), e.g. in lauryl- or oleoyl-sulphate, sodium deoxycholate, sodium glycodeoxycholate, sodium oleate, sodium taurate, a fatty acid salt, such as sodium elaidate, sodium linoleate, sodium laurate, a lysophospholipid, such as n-octadecylene(=oleoyl)-glycerophosphatidic acid, -phosphorylglycerol, or -phosphorylserine, n-acyl-, e.g. lauryl or oleoyl-glycero-
15 phosphatidic acid, -phosphorylglycerol, or -phosphorylserine, n-tetradecyl-glycero-phosphatidic acid, -phosphorylglycerol, or - phosphorylserine, a corresponding palmitoeloyl-, elaidoyl-, vaccenyl-lysophospholipid or a corresponding short-chain phospholipid, or else a surface-active polypeptide.

20 68. The method according to claims 54 to 67,
characterised in that the average diameter of the penetrant is between 30 nm and 500 nm, more preferably between 40 nm and 250 nm, even more preferably between 50 nm and 200 nm and particularly preferably between 60 nm and 150 nm.

25 69. The method according to claims 54 to 68,
characterised in that the total dry weight of droplets in a formulation is 0.01 weight-% (w-%) to 40 w-% of total formulation mass, more preferably between 0.1 w-% and 30 w-%, and most preferably between 0,5 w-% and 20 w-%.

70. The method according to claims 54 to 69,
characterised in that the total dry weight of droplets in a formulation is selected
to increase the formulation viscosity to maximally 200 mPas, more preferably up
5 to 40 mPas, and most preferably up to 8 mPas.

71. The method according to claims 54 to 70,
characterised in that at least one edge-active substance or surfactant and/or at
least one amphiphilic substance, and / or at least one hydrophilic fluid and the
10 agent are mixed, if required separately, to form a solution, the resulting (partial)
mixtures or solutions are then combined subsequently to induce, preferably by
action of mechanical energy such as shaking, stirring, vibrations, homogenisation,
ultrasonication, shearing, freezing and thawing, or filtration using convenient
driving pressure, the formation of penetrants that associate with and / or
15 incorporate the agent

72. The method according to claim 71,
characterised in that said amphiphilic substances are dissolved in volatile
solvents, such as alcohols, especially ethanol, or in other pharmaceutically
20 acceptable organic solvents, such as ethanol, 1- and 2-propanol, benzyl alcohol,
propylene glycol, polyethylene glycol (molecular weight: 200-400 D) or glycerol,
other pharmaceutically acceptable organic solvents, such as undercooled gas,
especially supercritical CO₂, which are then removed, especially by evaporation or
dilution, prior to making the final preparation.

25 73. The method according to any one of claims 68 or 72,
characterised in that the formation of said penetrants is induced by the addition
of required substances into a fluid phase, evaporation from a reverse phase, by
injection or dialysis, if necessary under the influence of mechanical stress, such as

shaking, stirring, especially high velocity stirring, vibrating, homogenising, ultrasonication, shearing, freezing and thawing, or filtration using a convenient, especially low (1 MPa) or intermediate (up to 10 MPa), driving pressure.

5 74. The method according to claim 73,
characterised in that the formation of said penetrants is induced by filtration, the filtering material having pores sizes between 0.01 μm and 0.8 μm , more preferably between 0.02 μm and 0.3 μm , and most preferably between 0.05 μm and 0.15 μm , whereby several filters may be used sequentially or in parallel.

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 75. The method according to any one of claims 55 to 74,
characterised in that said agents and penetrants are made to associate, at least partly,
– after the formation of said penetrants, e.g. after injecting a solution of the drug
15 in a pharmaceutically acceptable fluid, such as ethanol, 1- and 2-propanol, benzyl alcohol, propylene glycol, polyethylene glycol (molecular weight: 200-400 D) or glycerol into the suspending medium,
– simultaneously with penetrant formation , if required using the drug co-solution and, at least some, penetrant ingredients.

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 76. The method according to any one of the claims 55 to 75,
characterised in that said penetrants, with which the agent is associated, are prepared immediately before the application of the formulation, if convenient, from a suitable concentrate or a lyophilisate.

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 77. The method according to any one of the claims 55 to 76,
characterised in that the formulation is applied by spraying, smearing, rolling or sponging on the application area, in particular by using a metered sprayer, spender, roller or a sponge, or a non-occlusive patch, as appropriate.

78. The method according to any one of the claims 55 to 77,
characterised in that the barrier is skin or at least partly keratinised endothelium
and / or nasal or any other mucosa.

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79. The method according to claim 78,
characterised in that, the area dose of said penetrant is between 0.1 mg per
square centimetre (mg cm^{-2}) and 40 mg cm^{-2} , more preferably is between
 0.25 mg cm^{-2} and 30 mg cm^{-2} and even more preferably is between 0.5 mg cm^{-2}
and 15 mg cm^{-2} , in the case that the penetrant is applied on said skin and / or said
at least partly keratinised endothelium.

10

80. The method according to claim 78,
characterised in that the area dose of said penetrant is between 0.05 mg per
square centimetre (mg cm^{-2}) and 20 mg cm^{-2} , more preferably is between
 0.1 mg cm^{-2} and 15 mg cm^{-2} and even more preferably is between 0.5 mg cm^{-2} and
 10 mg cm^{-2} , in the case that the penetrant is applied on said nasal or other
mucosa.

15

81. The method according to claim 78,
characterised in that the area dose of said penetrant is between 0.0001 mg per
square centimetre (mg cm^{-2}) and 0.1 mg cm^{-2} , more preferably is between
 $0.0005 \text{ mg cm}^{-2}$ and 0.05 mg cm^{-2} and even more preferably is between 0.001 mg
 cm^{-2} and 0.01 mg cm^{-2} , in the case that the penetrant is applied on plant body,
plant leaves or plant needles.

20

25

82. The method of claim 54, used for generating an immune response
on a human or other mammal by vaccinating said mammal.

83. The method of claim 54, used for generating a therapeutic effect in a human or other mammal.

84. The method of claim 54 for the treatment of inflammatory disease,
5 dermatosis, kidney or liver failure, adrenal insufficiency, aspiration syndrome, Behcet syndrome, bites and stings, blood disorders, such as cold-haemagglutinin disease, haemolytic anemia, hypereosinophilia, hypoplastic anemia, macroglobulinaemia, trombocytopenic purpura, furthermore, for the management
10 of bone disorders, cerebral oedema, Cogan's syndrome, congenital adrenal hyperplasia, connective tissue disorders, such as lichen, lupus erythematosus, polymyalgia rheumatica, polymyositis and dermatomyositis, epilepsy, eye disorders, such as cataracts, Graves' ophthalmopathy, haemangioma, herpes infections, neuropathies, retinal vasculitis, scleritis, for some gastro-intestinal disorders, such as inflammatory bowel disease, nausea and oesophageal damage,
15 for hypercalcaemia, infections, e.g. of the eye (as in infections mononucleosis), for Kawasaki disease, myasthenia gravis, various pain syndromes, such as postherpetic neuralgia, for polyneuropathies, pancreatitis, in respiratory disorders, such as asthma, for the management of rheumatoid disease and osteoarthritis, rhinitis, sarcoidosis, skin diseases, such as alopecia, eczema, erythema
20 multiforme, lichen, pemphigus and pemphigoid, psoriasis, pyoderma gangrenosum, urticaria, in case of thyroid and vascular disorders.

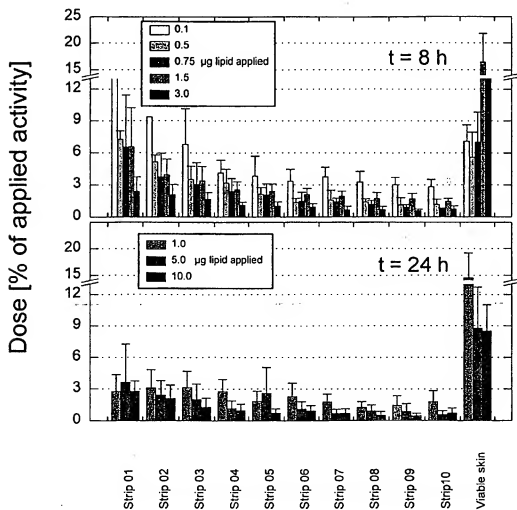


Figure 1

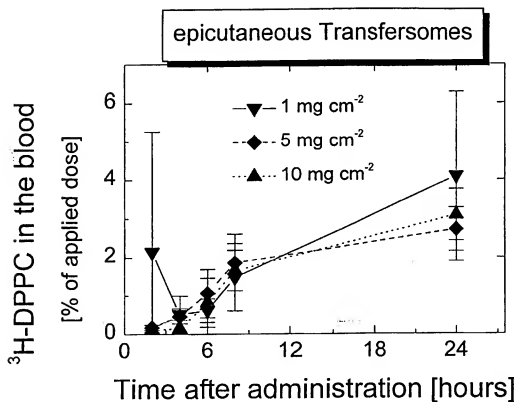


Figure 2

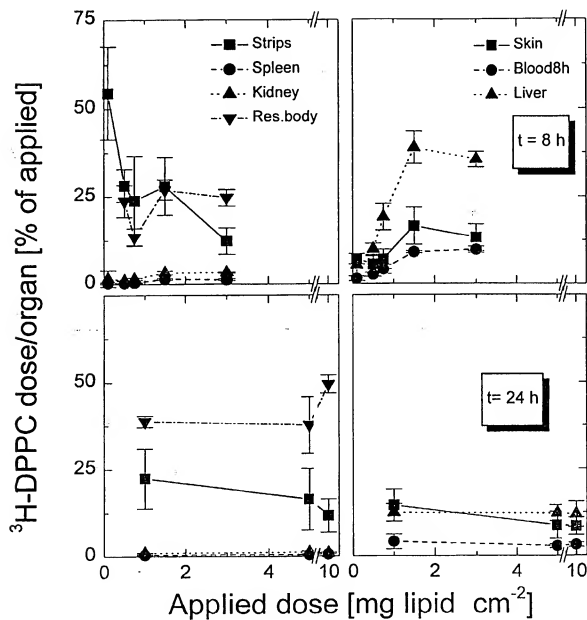


Figure 3

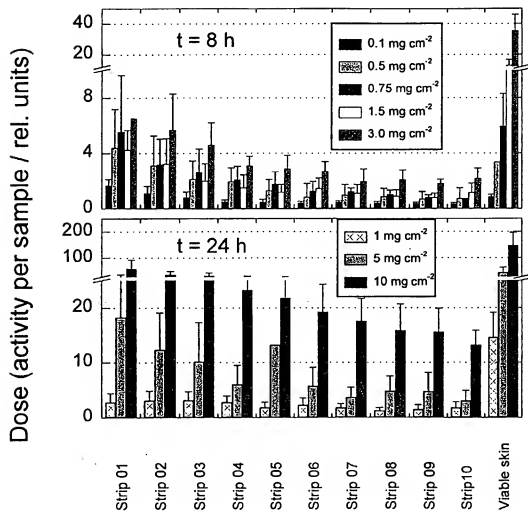


Figure 4

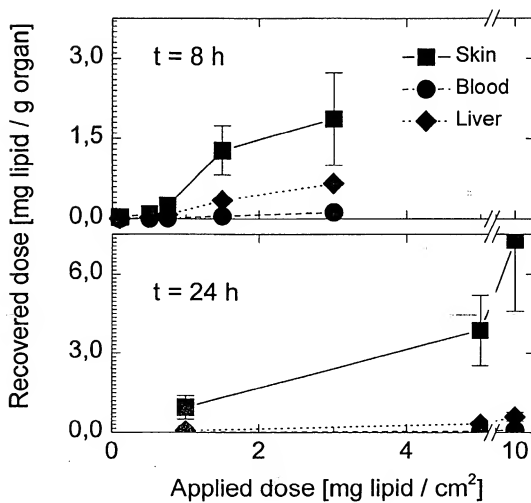


Figure 5

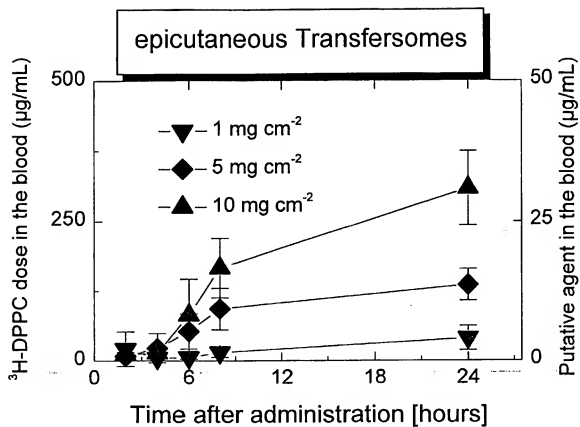


Figure 6

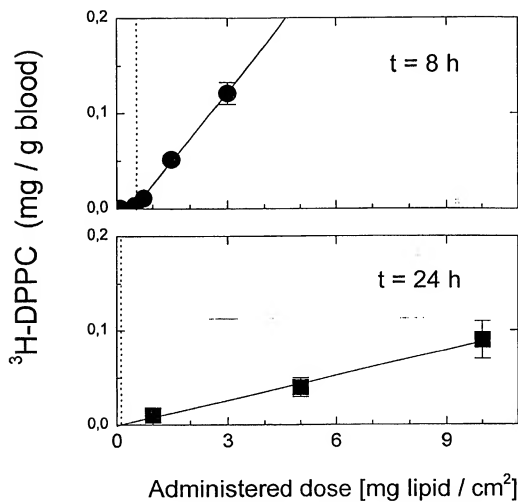
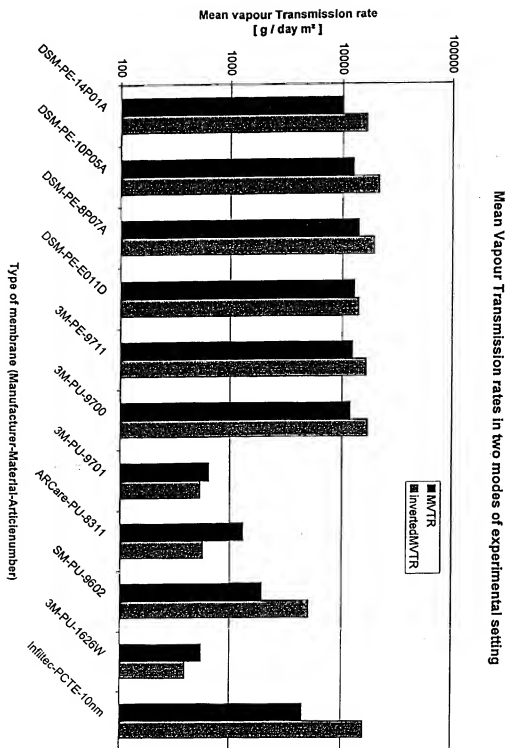


Figure 7

Figure 8



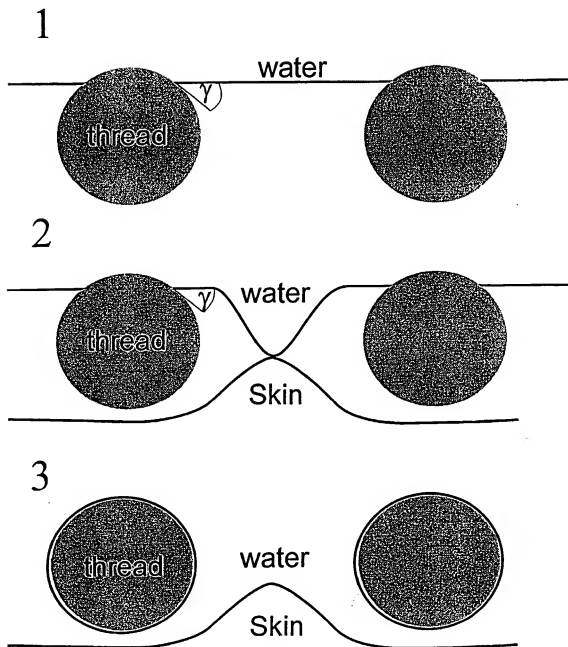
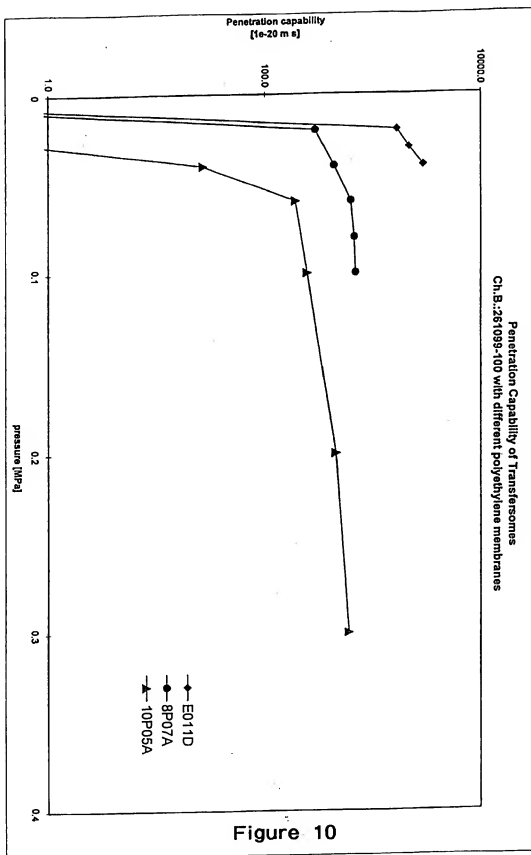


Figure 9



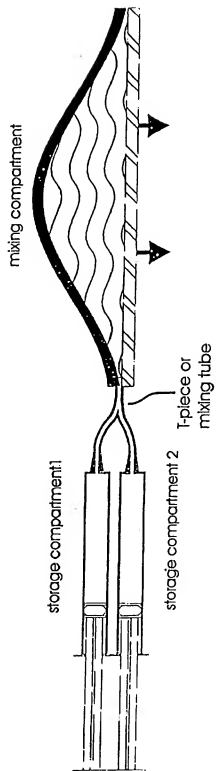


Figure 11

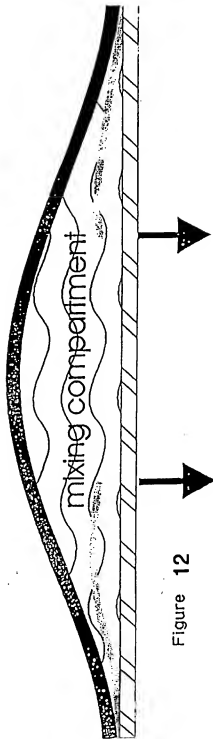
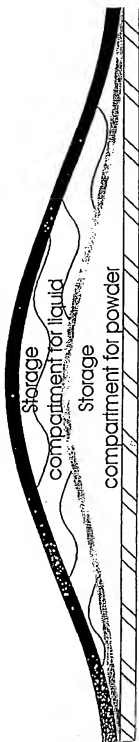


Figure 12

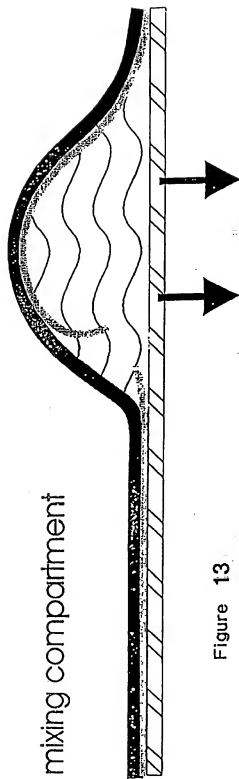
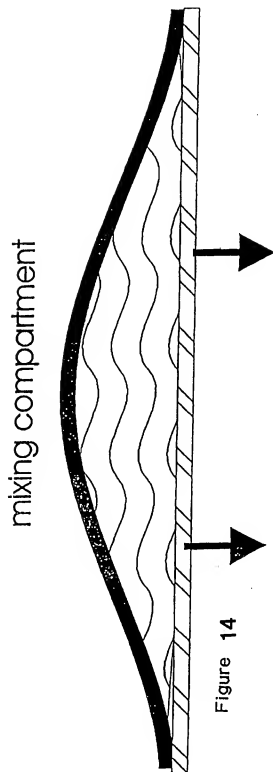
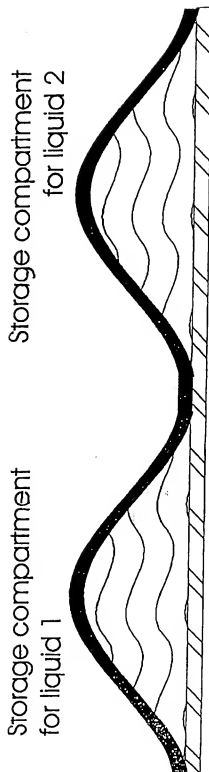


Figure 13



INTERNATIONAL SEARCH REPORT

Internat. Application No.
PCT/EP 00/06367A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K9/127 A61K9/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

WPI Data, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	G. CEVC ET AL.: "transfersomes-mediated transepidermal delivery improves the regiospecificity and biological activity of corticosteroids in vivo" JOURNAL OF CONTROLLED RELEASE, vol. 45, no. 3, 7 April 1997 (1997-04-07), pages 211-226, XP000640528 Amsterdam (nl) page 211, abstract page 225, conclusions page 213, paragraph 2.1. --- --/--	1,2, 12-15, 18-29, 54,55, 65-75, 78-81, 83,84

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

16 November 2000

Date of mailing of the international search report

24/11/2000

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Authorized officer

Benz, K

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 00/06367

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WO 98 17255 A (CEVC) 30 April 1998 (1998-04-30)</p> <p>the whole document page 19, line 21 - line 25 page 21, line 16 -page 23, line 17 page 24, line 9 - line 25 page 27, line 4 -page 28, line 4 claim 18</p>	<p>1-28, 54-57, 61,63, 65-69, 71-76, 78-84</p>
A	<p>V.M. KNEPP ET AL.: "controlled drug release from a novel liposomal delivery system. II. transdermal delivery characteristics" JOURNAL OF CONTROLLED RELEASE, vol. 12, no. 1, March 1990 (1990-03), pages 25-30, XP000113393 Amsterdam (NL) page 26, column 1, paragraph 6. page 26, column 2, paragraph 2</p>	29-32
A	<p>EP 0 674 913 A (LECTEC CORPORATION) 4 October 1995 (1995-10-04) the abstract</p>	1, 29-35
A	<p>WO 98 30215 A (CILAG) 16 July 1998 (1998-07-16) claims 1-9</p>	1, 4-11

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 00/06367

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9817255 A	30-04-1998	AU 724218 B AU 4510897 A BR 9612750 A EP 0935457 A HU 9903363 A	14-09-2000 15-05-1998 28-12-1999 18-08-1999 28-03-2000
EP 674913 A	04-10-1995	US 5536263 A AU 676623 B AU 1002495 A CA 2133598 A FI 950465 A JP 7265353 A NO 951217 A US 6096334 A US 5741510 A US 6096333 A	16-07-1996 13-03-1997 12-10-1995 01-10-1995 01-10-1995 17-10-1995 02-10-1995 01-08-2000 21-04-1998 01-08-2000
WO 9830215 A	16-07-1998	AU 5777498 A	03-08-1998



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification⁴ : A61K 9/42, A61J 5/00 B01J 13/02, B32B 5/16</p>	<p>A1</p>	<p>(11) International Publication Number: WO 87/ 01938</p> <p>(43) International Publication Date: 9 April 1987 (09.04.87)</p>
<p>(21) International Application Number: PCT/US86/01893</p> <p>(22) International Filing Date: 11 September 1986 (11.09.86)</p> <p>(31) Priority Application Number: 781,147</p> <p>(32) Priority Date: 27 September 1985 (27.09.85)</p> <p>(33) Priority Country: US</p> <p>(71) Applicant: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 2199 Addison Street, Berkeley, CA 94720 (US).</p> <p>(72) Inventors: GUY, Richard, H. : 342 Ardenale Drive, Daly City, CA 94014 (US). SZOKA, Francis, C. : 45 Mendosa Avenue, San Francisco, CA 94116 (US). KNEPP, Victoria, M. : 375 Carl Street, Number 4, San Francisco, CA 94117 (US). WESTER, Ronald, C. : 8 Lockoley Avenue, 2D, San Francisco, CA 94122 (US).</p>	<p>(74) Agents: DEHLINGER, Peter, J. et al.; Ciotti & Murashige, 545 Middlefield Road, Suite 200, Menlo Park, CA 94025 (US).</p> <p>(81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).</p> <p>Published <i>With international search report.</i></p>	
<p>(54) Title: LIPOSOME TRANSDERMAL DRUG DELIVERY SYSTEM</p>		
<p>(57) Abstract</p>		
<p>A transdermal drug release device for releasing a physiologically active, lipophilic drug to a skin surface at a selected, substantially constant rate over a period of at least about 24 hours. The device includes an aqueous-phase matrix having a surface adapted to be placed against the skin surface, and, embedded within the matrix, liposomes containing the drug in entrapped form and composed of lipid components which are selected to produce such drug release rate. In one embodiment, the matrix contains a water-soluble drug in free form, and then entrapped lipophilic drug is a drug transport agent designed to facilitate transdermal uptake of the water-soluble drug.</p>		

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LIPOSOME TRANSDERMAL DRUG DELIVERY SYSTEM1. Field of the Invention

The present invention relates to transdermal
5 drug delivery devices.

2. Background of the Invention

The delivery of drugs via the skin to produce
systemic effect is attractive for many reasons.
10 Depending on the site of transdermal drug
administration, first-pass metabolism by the liver may
be avoided. Often the drug can be administered close to
the site of action, allowing a more localized drug
action. Because a steady, sustained concentration of
15 drug at the site of action can be maintained, dosing
frequency and dose magnitude can be reduced, with fewer
side effects and better patient compliance.

A variety of transdermal drug delivery devices
have been proposed heretofore. One general type of
20 transdermal device consists of a matrix reservoir
containing a drug in free form, and one or more
microporous walls which limit the rate of drug diffusion
out of the matrix. For example, United States Patent
No. 4,336,243 describes a nitroglycerine patch in which
25 the drug is contained in a cross-linked silicon matrix
formed of microporous, cross-linked walls. In the
transdermal devices disclosed in United States Patents
Nos. 4,286,592, 4,060,084, 3,371,683, and 3,596,122, the
rate of drug release from the matrix is controlled by a
30 drug release barrier placed between the matrix and skin.

Transdermal devices of the type just described
have a number of limitations. The rate of drug
diffusion through the microporous wall can vary widely,
depending on drug size and solubility. Therefore,

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considerable design work may be required to construct a diffusion barrier having desired permeability properties for a given drug. Also, where the diffusion barrier is a single membrane, small variational defects in the membrane can lead to significant variations in drug release rate. The devices are best suited to water-soluble drugs which can be dispersed in high concentration throughout a hydrated matrix.

In another type of known transdermal drug delivery device, the drug is encapsulated in microcapsules which are distributed throughout a drug matrix. The microcapsule walls are permeable to passage of the drug, and act to control the rate of drug release from the device. Devices of this types are disclosed in U.S. Patents Nos. 3,598,123, 3,797,494, and 3,464,413. Typically the drug is a water-soluble drug encapsulated in a polymer-shell microcapsule.

As in the diffusion-barrier devices mentioned above, considerable design work may be required to discover the proper material and cross-linking conditions which produce a microcapsule with the desired permeability characteristics for a given drug. Further, drug encapsulation and diffusion-barrier control are generally impractical for lipophilic drugs.

3. Summary of the Invention

It is a general object of the invention to provide an improved transdermal drug release device which overcomes above-mentioned limitations in the prior art.

A specific object of the invention is to provide such a device for releasing a lipophilic drug at a selected, substantially constant release rate over a period of at least about 24 hours.

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Another object of the invention is to provide a transdermal device for delivering a water-soluble drug under conditions which promote transdermal uptake of the drug.

5 Still another object of the invention is to provide a method of achieving a selected, substantially constant release rate of a lipophilic drug in a transdermal device.

10 The liposome drug release device, or patch of the invention includes an aqueous-phase matrix having a surface adapted to be placed against the skin surface. Liposomes embedded within the matrix and containing an entrapped lipophilic drug are formulated to produce a selected, substantially constant rate of drug release
15 for a period of at least about 24 hours. The concentration of liposomes in the matrix is preferably between about 5 and 25 percent by volume, and the major portion of drug in the matrix is partitioned initially in the liposome bilayers.

20 In one embodiment, the matrix contains a water-soluble drug in free form, and the entrapped lipophilic drug is a drug-transport agent effective to facilitate transdermal uptake of the water-soluble drug. The device may include a diffusion barrier to
25 control the rate of release of water-soluble drug from the matrix, such that both the water-soluble drug and uptake agent are delivered from the device at relatively constant, selected release rates.

30 In another aspect, the invention includes a method of achieving a selected, substantially constant rate of release of a lipophilic drug from an aqueous-phase matrix over a period of at least about 24 hours. The method involves entrapping the drug in liposomes containing phospholipid components which are

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selected to produce such a rate of drug release from the liposomes into an aqueous medium, and embedding the liposomes in the matrix.

These and other objects and features of the present invention will become more fully apparent from the following detailed description of the invention, when read in conjunction with the accompanying drawings.

Brief Description of the Drawings

Figure 1 is a schematic sectional view of a transdermal drug delivery patch constructed according to one embodiment of the invention;

Figure 2 is a sectional view of a transdermal drug delivery patch constructed according to another embodiment of the invention;

Figures 3A and 3B are, respectively, graphs of percent progesterone (PG) release per hour in a transdermal patch containing free PG, over a 24 hour period, and cumulative percent PG released from the patch over the same time period;

Figures 4A and 4B are graphs showing PG released per hour and cumulative PG released, respectively, for a transdermal patch in which PG is entrapped in egg phosphatidylcholine (PC) liposomes;

Figures 5A and 5B are graphs showing PG released per hour and cumulative PG released, respectively, for a transdermal patch in which PG is entrapped in dipalmitoyl phosphatidylcholine (DPPC) liposomes;

Figures 6A and 6B are graphs showing PG released per hour and cumulative PG released, respectively, for a transdermal patch in which PG is contained in oil emulsion particles;

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Figure 7 is a plot of the relationship between percent PG release and square root of release time for an egg PC liposome transdermal patch;

Figures 8A and 8B are graphs showing respectively, the percent dose of PG per hour and cumulative PG delivered across full-thickness skin, for a transdermal patch in which PG is contained in free form;

Figures 9A and 9B are graphs showing, respectively, the percent dose of PG per hour and cumulative PG delivered across full-thickness skin, for a transdermal patch in which PG is entrapped in egg PC liposomes; and

Figures 10A and 10B are like Figures 9A and 9B, respectively, for a patch in which PG is entrapped in DPPC liposomes.

Detailed Description of the Invention

1. Liposome Transdermal Patch

The transdermal drug-release device, or patch, of the invention includes an aqueous-phase matrix, and embedded within the matrix, liposomes containing a lipophilic drug in entrapped form. The liposomes are composed of lipid components and preferably phospholipid components which are selected to produce a desired rate of drug release from the matrix. According to an important aspect of the invention, the drug-release rate is substantially constant for at least about 24 hours, and up to two weeks or more, depending on the liposome composition.

The lipophilic drug may be a systemically active drug which is effective at relatively low blood levels, when delivered over a prolonged period. Such

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drugs include birth control steroids, such as mestranol, norethynodrel, ethanyl estradiol, norgestrel, estradiol, progesterone, and norethindrone; other steroids, such as testosterone; anti-inflammatory drugs, such as cortisone and triamcinolone, analgesic drugs, such as morphine and codeine; nitrates, such as nitroglycerine and isosorbide dinitrate; anti-hypertensives, such as furosamide, chlorothiazide, minoxidil and propranol; anti-depressants, such as protriptyline; cardiovascular agents, such as quinidine, disopyramide, and clonidine; fat-soluble vitamins, such as vitamin A and D; prostaglandins; prostacyclines; and calcium channel blockers, such as nifedipine.

Alternatively, the drug may be one whose primary site of action is the site of topical administration. Drugs of this type include anti-inflammatory agents, antibiotics, analgesics, and local anesthetics, such as novacaine, and lidocaine.

In another general embodiment of the invention to be described below, the lipophilic drug is a drug-transport agent designed to facilitate transdermal uptake of a water-soluble drug which is present in free form in the matrix and delivered from the matrix concurrently with the drug-transport agent. Exemplary transport agents include a variety of aliphatic, cycloaliphatic and aromatic compounds, such as those named in U.S. Patent No. 3,797,494. One preferred type of drug-transport agent is selected from a class of azacyclo pentane- and heptane-ones, such as those disclosed in U.S. Patents Nos. 4,316,893 and 3,989,816.

According to an important feature of the invention, the rate of drug release from the matrix is controlled by the rate of drug exchange between liposomes and the surrounding aqueous medium. In

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particular, studies conducted in support of the invention, and reported in Section II below, show that liposomes have the capability of releasing (exchanging) drug at a relatively constant rate, independent of liposome drug concentration, and that the rate of drug release, or exchange, can be controlled, within broad limits, by varying the lipid composition of the liposomes.

In order to achieve liposomal control of drug release, it is necessary that a major portion of the drug in the matrix be partitioned initially in the lipid phase of the embedded liposomes. Partitioning of the drug between the lipid (liposome bilayer) and aqueous phase (the non-liposomal bulk aqueous phase) is determined by the lipid/aqueous partition coefficient of the drug and the volume percent of liposomes in the matrix. For example, in a device in which the liposomes constitute 10 percent of the total matrix volume, a drug having a liposome/aqueous phase partition coefficient of 10 will partition in roughly equal amounts between the liposomes and bulk aqueous phase of the matrix. At a drug partition coefficient of 1000, about 99% of the drug will be entrapped. The partition coefficient of the drug and the volume percent of liposomes in the matrix are preferably selected to insure that at least about 50% and more preferably, 80% or more of the drug is contained in the liposomes at initial drug-release conditions. Assuming that the liposomes make up, at most, about 25% of the matrix volume, the lipophilic drug used in the invention should therefore have a partition coefficient of at least about 4.

The partition coefficients of many drugs have been determined for two-phase oil water solvent mixtures, such as an octanol/water mixture (see, for

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example, Yalkowsky, S.H., et al, Pharma. Sci. 72:866 (1983)). The drug partition coefficients determined using simple oil/water mixtures are useful in comparing the relative lipophilic character of different drugs.

5 However, the actual partition coefficient of a drug in liposomes depends on several factors which affect the hydrophobicity of the liposome bilayer region and degree of lipid packing in the liposome bilayer. Although actual liposome/water partition coefficients for

10 selected liposomes can be measured, it is usually simpler to measure directly the drug release rates of a selected drug with respect to liposomes having different lipid compositions, and at different liposomes concentrations, and from these measurements, to

15 determine (a) if the drug is sufficiently lipophilic to show liposome-controlled drug release and (b) if so, the liposome composition and concentration which will produce the desired drug release rate in the device.

Considering the selection of suitable liposome components, co-owned U.S. patent application for

20 "Liposome Inhalation System and Method", Serial No. 737,221, filed 25 March 1985, describes an in vitro system for determining the rate of release of liposome-entrapped drugs, as a function of liposome

25 lipid composition. The system was used to examine 40 different phosphatidylcholine (PC) lipids and lipid mixtures to determine the effect of acyl chain length, degree of saturation, polar head group charge, and presence of cholesterol on the release of metaproteranol

30 sulfate (MPS) from liposomes. Although MPS is a relatively water-soluble drug, studies conducted in support of the present invention, and reported in Example III below, indicate that the general finding for MPS also apply to a highly lipophilic drug, such as

-9-

progesterone (PG). Briefly, it has been found with both drug types that the most important compositional factor for drug release rates is phospholipid acyl chain length and degree of saturation, with more fluid acyl chains (shorter and/or more unsaturated) giving higher release rates. Thus, in Example III it is seen that liposomes formed with egg PC, which includes a mixture of relatively short-chain and unsaturated lipids, have a drug release rate about six times higher than that of liposomes formed with dipalmitoylphosphatidylcholine (DPPC), a relatively long-chain, saturated lipid. Pure phospholipids and phospholipid mixtures with a variety of acyl chain moieties are commercially available or can be isolated or synthesized by known methods.

The effect of cholesterol on drug release rate in egg PC liposomes has also been examined (Example III). Liposomes containing PC and cholesterol, at a 2:1 mole ratio, showed substantially the same PG release rate as did pure egg PC liposomes (about 1% total drug release per hour). These results are consistent with earlier studies on release of MPS from liposomes, which showed that cholesterol only slightly increased drug release in egg PC liposomes. However, the MPS studies did show that cholesterol can significantly increase drug-release in liposomes whose phospholipid components contain long-chain, saturated acyl groups.

The lipids forming the liposomes may, of course, include phospholipids and sterols other than the model lipids used in the above-mentioned studies and Examples, and may also include other types of lipids, such as glycolipids and sphingolipids, which are compatible with liposome formation. A list of lipids which are used commonly in liposome formation is given on page 471 of Szoka, F., Jr., et al, Ann. Rev. Biophys.

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Bioeng. 9:467 (1980). The liposomes may also be formulated to include various types of drug-protective or lipid-protective agents, such as the antioxidant α -tocopherol, which was included at a 0.6 mole percent in the liposomes described in the examples.

The liposomes may be prepared by a variety of techniques, such as those detailed in the above-cited Szoka et al reference. A simple lipid-hydration procedure for producing multilamellar vesicles (MLVs) is generally suitable. In this procedure, a mixture of vesicle-forming lipids and the drug are dissolved in a suitable organic solvent or solvent mixture, then evaporated in a vessel to form a thin film. Hydrating the thin film with an aqueous solution, over a period of at least about 1 hour, produces multilamellar vesicles (MLVs), with sizes typically between about 0.1 to 10 microns.

Although the drug is preferably incorporated initially into the lipids forming the vesicles, as above, it may also be diffused into preformed liposomes. If necessary, the drug diffusion step can be carried out in the presence of a mild detergent, such as deoxycholate, to facilitate drug exchange with the liposomes. The detergent can be removed by dialysis after liposome uptake.

The amount of drug which is included in the lipid-forming vesicles is that calculated to produce a selected total drug concentration in the transdermal device. The amount of drug that can be incorporated into the transdermal device is limited by the amount of drug that can be stably incorporated into liposomes, the maximum allowed concentration of liposomes in the matrix and patch size. Typically, vesicle-forming lipids can form stable liposomes in the presence of between 1-5

-11-

mole percent drug. For some drugs, such as a variety of sterols, the drug concentration can be as high as 40-50 mole percent without significant membrane instability. In general, the drug to lipid ratio can be safely increased until an increase in the rate of drug release is observed in the liposomes. The increase drug-release rate signals drug-saturation of the liposomes and membrane instability.

The maximum concentration of liposome which can be embedded in the matrix will depend on the nature of the material forming the matrix and the method of matrix formation. Generally, the concentration of liposomes in the matrix will be between about 5% and 25% based on the total liposome volume per total matrix volume.

The matrix can be formed of any dermatologically acceptable gel or cross-linked polymer material which is compatible with an aqueous medium, and capable of gelling or forming cross links under conditions, particularly temperature conditions, which do not disrupt liposomes. A variety of polymers and polymer mixtures, which are suitable are listed in U.S. Patent No. 3,797,494, columns 14 and 15. Also disclosed in this patent are a number of gel-forming polymers, such as cellulose derivatives, which are useful in forming the matrix. One preferred matrix material used in the device described in Examples II-IV, is the polysaccharide agarose.

To form the matrix, liposomes are added to an aqueous suspension or solution of the matrix forming material, and the mixture is then cross-linked or gelled to produce a rigid or semi-rigid matrix structure having immobilized liposomes embedded throughout. In the case of a polymer type matrix material, the cross-linking step may involve adding a chemical catalyst, and/or

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light-induced cross-linking. For example, an acrylamide polymer matrix can be formed by cross-linking acrylamide, either by addition of a free-radical generator such as ammonium persulfate, or by light-induced cross-linking in the presence of a photosensitizer such as riboflavin. Methods for cross-linking polymers under suitable temperature conditions are well known. The material is cross-linked in a suitable mold.

A gel matrix is formed typically by heating gel material in an aqueous medium to its solution temperature, cooling the solution to somewhat above its gelling temperature then mixing the material with a selected volume of liposome suspension. The concentration of the gelled material is selected to provide a relatively firm, shape-retaining structure after dilution with the liposome suspension. Typically, the final gel concentration is between about 1%-5% w/v. The liposome/gel material is poured into a mold and cooled to form the matrix. Example II below describes the preparation of liposome patches by mixing a 4% agarose solution at 67°C with an equal volume of liposome suspension and allowing the mixture to gel by slow cooling.

The matrix is preferably covered on one side by an impermeable cover, such as metal foil or the like, to prevent drying out during use. The cover can be attached by adhesives or the like after matrix formation. Alternatively, the cover can be bonded to the matrix during the molding step, by pouring the still-liquid matrix material over the cover in a mold.

Figure 1 is a schematic, sectional view of a liposome drug-delivery device 10 prepared according to the invention. An aqueous-phase matrix, indicated at

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12, contains liposomes, such as those shown at 14, embedded throughout. The liposomes serve as a reservoir for a lipophilic drug, a portion of which is available in free form, as indicated at 18. The lower surface of the matrix in the figure defines a drug-release surface 20 adapted to be placed against a skin surface for transdermal drug delivery. The upper surface is sealed with an impermeable cover 22. Although not shown, the cover extends beyond the edges of the matrix, and is adhesive backed in its extending edge regions, allowing the device to be adhesively attached to a skin surface.

An alternative embodiment of the invention, for use in delivering a water-soluble drug, is illustrated at 24 in Figure 2. In operation, this device releases both a water-soluble drug, which is contained in free form in the matrix, and a lipophilic drug-transport agent of the type mentioned above, which acts to increase transdermal uptake of water-soluble molecules. The transport agent is contained in liposomes, such as those at 26, and these are embedded in an aqueous-phase matrix 28, as in device 10 above. The device is formed as above, except that the water-soluble drug, indicated at 29, is included in the polymer or gel material used in forming the matrix. Exemplary water-soluble drugs include antihistamines, such as dimenhydrinate, diphenhydramine, chlorpheniramine, and ephedrin; anti-motion drugs, such as scopolamine; antibiotics, such as penicillin; and tranquilizers and other nervous disorder drugs, such as dopa.

Device 24 has a cover 30 protecting its upper surface against dehydration, and also has a diffusion-barrier membrane 32 covering its lower drug-release surface. Membrane 32 functions to limit the rate of release of the water-soluble drug from the

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matrix, preferably at a release level which is compatible with the release rate of the lipophilic drug-transport agent. The diffusion-limiting membranes described in U.S. Patents Nos. 4,286,592; and 3,598,123, for use in limiting the rate of passage of water-soluble membranes are generally suitable.

III. Drug-Release and Uptake Characteristics

This sections examines the drug-release characteristics of liposome transdermal devices and the ability of the devices to modulate drug uptake by skin. Drug release rates were measured as detailed below in Example III. Briefly, a PG-liposome patch, prepared as in Section I, was placed in one chamber of a two-chamber diffusion cell. The second confronting chamber was filled with a suitable receptor liquid to serve as a reservoir for released drug. Receptor fluid was flowed through the reservoir at a suitable rate, and the reservoir discharge sampled every hour for released radioactive PG. Transdermal patches containing PG in three different liposome formulations and in free and emulsion form were compared. One liposome formulation containing egg PC, whose fatty acyl composition is 16:0 (42%), 18:1 (28%), and 18:2 (16%), i.e., both relatively short (16:0) and unsaturated (18:1 and 18:2) acyl chains. A second liposome formulation contained egg PC/cholesterol, at a 2:1 mole ratio. The third formulation contained pure DPPC, whose palmitoyl chains (18:0) are relatively long and unsaturated.

Figure 3A shows the levels of drug release from the free-drug patch measured at 1 hour intervals over a 24 hour period, where the radioactivity measured at each time point is expressed as percent of total radioactivity in the patch. As seen from the figure,

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about 20% of the total free drug was released in the first hour, and drug release levels declined sharply over the first 15 hours. When the data is plotted as cumulative percent dose, the curve shown in Figure 3B is obtained. As seen, nearly 90 percent of the free drug is released in the first 24 hours.

Drug release characteristics of a liposome patch prepared with egg PC lipids are shown in Figure 4A and 4B. Referring to Figure 4A, the liposome patch released progesterone at a substantially constant release rate of about 1 percent total drug per hour over the 24 hour test period. The plot of cumulative amount released over the 24 hour period (Figure 4B), shows a substantially linear increase in released PG over 24 hours, to about 25% of total drug. Drug-release measurements on the egg PC/cholesterol patch, taken every hour over a 48 hour test period were similar to those for egg PC liposomes. The rate of drug release was 1% per hour over the test period, and about half the drug was released in 48 hours.

The drug release characteristics of the the DPPC liposome patch were also measured over a 48 hour period, with the results shown in Figures 5A and 5B. The percent dose release every hour, plotted in Figure 4A, remained substantially constant, at between about 0.15 and 0.2 percent total drug, over the entire test period. The cumulation of drug dose, plotted in Figure 5B, shows that (a) the drug accumulates in a linear fashion, and (b) the rate of accumulation is about 4% total PG every 24 hours. The drug accumulation rate is that seen for both the egg PC and egg PC/cholesterol liposome patches.

The drug release and accumulation characteristics of a lipid emulsion patch are graphed in

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Figures 6A and 6B, respectively. With reference to Figure 6A, the drug release rate was substantially constant at about 2.5% per hour, for the first 8-10 hours, then declined to about 1% per hour after 24 hours. As seen in Figure 6B, the released drug reached an accumulated level of about 30% of total drug during the first 12 hour period of linear drug release, and a final accumulated drug level of about 50% after 24 hours. In Figures 3A-6A, the error bars are standard deviations for 5-6 individual tests.

The data from Figures 3-6 are summarized in Table I below. The lipid composition in the patch tested is indicated at the left in the table. The two middle columns show the approximate drug release rate/hour, expressed as percent total drug, and the time over which the drug release rate is substantially linear. The final column shows the cumulative drug levels after 24 hours.

Table I

Lipid	Drug Release Rate/Hour	Linear (hours)	Cumulative (24 hours)
Free PC	--	--	90%
Emulsion	2.5%	8-10	50%
Egg PC	1%	>24	25%
Egg PC/CH	1%	>48	25%
DPPC	0.16%	>48	4%

The drug release curves in Figures 3B-6B were compared with theoretical drug release curves predicted from mathematical modeling, using Fisk's second law of diffusion with appropriate boundary conditions. A

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linear cumulative percent dose vs time, as was observed for the liposome drug release patches, is consistent with a model in which drug release in the system is modulated by slow interfacial transfer from the lipid bilayer into the aqueous receptor phase, rather than by diffusion through the matrix. According to this model, the liposomes act as a reservoir of drug within the matrix, with drug release from the reservoir being controlled by the partitioning of the drug in the matrix rather than by total drug concentration. By contrast, classic release behavior involving matrix diffusion predicts that the total percent drug release will be directly proportional to the square root of time, i.e., the system will show a strong time dependence related to a decreasing drug concentration in the matrix. The percent release vs square root of time for the EPC liposome patch is plotted in Figure 7. The linear relationship expected for a matrix diffusion process was not observed.

The ability of the above liposome transdermal patches to modulate transdermal drug uptake across full-thickness skin was also examined. Drug uptake through the skin was measured by placing a piece of full-thickness mouse skin between the chambers in a two chamber diffusion cell, and measuring release of drug (PG) into a flow-through reservoir, as above. The study compared drug uptake by skin from patches containing either free PG, PG in egg PC liposomes, or PG in DPPC liposomes. Details of the method are given in Example IV.

Figure 8A shows the levels of PG delivered transdermally into the lower reservoir from the free-drug patch, measured at 1 hour intervals over a 48 hour period. The radioactivity measured at each time

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point is expressed as percent total radioactivity in the patch. As seen, the amount of drug penetrating the skin barrier reached a maximum of about 0.4%-0.5% within 6-8 hours, and remained at this level throughout the test period (only the first 40 hours of the test period are shown). The skin sample variation in drug-release rates is indicated by the standard deviation bars in the figure. When the drug-release data is plotted as cumulative percent dose, the curve in Figure 8B is obtained, showing that about 25% of the total drug penetrated the skin barrier in the 48-hour test period.

The rate of transdermal PG uptake, when the drug is released from an egg PC liposome patch, is shown in Figure 9A. The rate of drug transport across the skin reached a level of about 0.2% per hour within about 16 hours, then substantially plateaued over the next 40 hours. The plot of accumulated drug over time (Figure 9B), when extrapolated to 48 hours, showed that about 10% of the total drug was delivered over a 2-day period. The liposome patch thus modulates, by a factor of 2-3, the rate of transdermal drug uptake, compared with a diffusion-limited (free-drug) transdermal device.

It is known that transdermal uptake varies widely among individuals, and this variation probably accounts for the relatively large standard deviations observed among the 4 test-subject skin samples in Figure 8A for progesterone supplied by the free-drug patch. A comparison of Figures 8A and 9A shows that the liposome drug delivery patch, which supplies progesterone at a controlled, substantially constant rate, significantly reduces this variation.

The ability of a liposome transdermal device to modulate transdermal uptake and reduce individual-specific variations in drug uptake is seen more

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strikingly in Figures 10A and 10B, which plot transdermal uptake characteristics observed with a DPPC liposome patch. Figure 10A shows that transdermal uptake increased over the first 8-16 hours, then
5 plateaued over the next 24 hours at about 0.03% per hour, with a standard deviation of about .01% total dose. Total drug uptake was about 1.35% after 48 hours.

The results of the transdermal uptake studies are summarized in Table II below. The drug transport
10 rate is the steady-state level observed after several hours, and the standard deviation is an approximate average of the standard deviation observed during the steady-state period. The roughly five-fold difference in drug transport rates between the egg PC and DPPC
15 patches is consistent with the difference in the drug-release rates (Table I) between the two patches.

Table II

20	<u>Lipid</u>	<u>Drug Transport (rate/hr)</u>	<u>Standard Deviation</u>	<u>Cumulative (24 hr)</u>
	Free PC	0.4%	~.2%	25%
	Egg PC	0.2%	~.1%	10%
25	DPPC	0.03%	~.02%	1.35%

The ability of the liposome transdermal patch to control drug bioavailability in vivo has been confirmed in tests with rhesus monkeys. The tests were
30 designed to determine estradiol transdermal bioavailability by cumulative urine excretion following drug administration. The drug/liposome patch was applied for either 24 or 48 hours. The bioavailability was about 1% total drug for the 24 hour

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application and roughly twice that for the 48 hour application. In both cases, the drug level remained constant for 24 hours after the patch was removed. The same quantity of drug administered topically in free
5 form (as a topical solution) gave about 10% drug level after 24 hours and the drug level fell rapidly during the 24-48 hour period. These results confirm that (a) the liposome drug delivery device functions to deliver a drug transdermally at a controlled rate and (b) the
10 total amount of drug delivered increases linearly with time of application.

From the foregoing it can be appreciated how various objects of the invention are met. The liposome patch is easily constructed and the lipid and matrix
15 components are stable for periods of several months or more under suitable storage conditions.

The liposome system can be readily adapted to deliver any relatively stable, lipid-compatible lipophilic drug, and the drug release rate can be
20 readily adjusted by appropriate selection of liposome lipid components and liposome concentration. In addition, where the drug is charged, the salt concentration of the aqueous medium can also be adjusted to alter the partitioning of the drug in the matrix.

25 Since the drug-release rate is largely dependent on drug partitioning across a liposome/water interface, rather than on drug concentration within the liposomes, the device can be designed to produce a controlled, substantially constant rate of drug release
30 over an extended period. The studies on the DPPC liposome device indicate that a constant drug release for up to 2 weeks is possible. Further, the total amount of drug released, at a selected drug release

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rate, is easily varied by varying drug concentration in the liposomes or the volume of the patch.

The device is able to modulate the rate of drug transport through skin, and the data in Table II indicates that the extent of modulation can be controlled over at least a five-fold range, by selected changes in liposome composition.

The following examples describe several liposome transdermal drug delivery devices and their drug release characteristics. The examples are intended to illustrate, but not limit, the invention.

Materials

Phospholipids, at greater than 99% purity, were obtained from Sigma Chem Co (St. Louis, MO); cholesterol, from Sigma Chemical Co. (St. Louis, MO), and C¹⁴-labeled Progesterone (PG). INTRALIPID was supplied by Cutter Labs (Berkeley, CA). GELBOND backing, an impermeable membrane, and agarose were obtained from FMC Corporation (Rockland, ME).

Example I

Drug/Lipid Preparations

Lipid solutions containing either (a) pure egg PC, (b) egg PC:cholesterol, at a mole ratio of 2:1, or (c) pure DPPC were prepared to a final lipid concentrations of about 100 mg/ml in chloroform. Each of the solutions (2.5 ml) was combined with about 0.4 μ Ci C¹⁴PG in a round bottom flask and the mixture dried to a film under vacuum.

To each flask was added citrate buffered saline, pH 4.5, to a final lipid concentration of about 63 mg lipid/ml, and a final C¹⁴ PG concentration of about 0.1 μ Ci/ml. The lipid films were hydrated by

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gentle swirling for one hour at 37°C to form suspensions of multilamellar vesicles (MLVs). The suspensions are designated egg PC, egg PC/cholesterol, and DPPC, according to their lipid composition.

- 5 INTRALIPID, an emulsion of soybean oil in a physiological salt solution, was concentrated to 22 mg lipid/ml. C^{14} PG was added to the concentrated emulsion to a final concentration of about 0.5 μ Ci/ml.

Example II

10

Preparing Transdermal Patches

A suspension of agarose in citrate-buffered saline, pH 4.5, was solubilized by heating, then cooled to 67°C.

- 15 Transdermal liposome patches were prepared by mixing equal volumes of 4% agarose at 67°C and a selected MLV suspension from Example I, and pouring about 1 ml of the warm mixture into a 5 cm square mold. The bottom of each mold was covered with a 16 cm square
20 piece of GELBOND membrane, with the hydrophilic side of the membrane facing up. The agarose-lipid mixture was allowed to gel by cooling to room temperature. Each patch had a final thickness of about 1.5 mm, and contained a total of about 40 μ Ci C^{14} PG.

- 25 An INTRALIPID PATCH was similarly prepared by mixing 0.9 ml of agarose and 0.12 ml of INTRALIPID to a final agarose concentration of about 2%. The patch contained 38-40 μ mole lipid and 0.05 μ Ci C^{14} PG.

- 30 A transdermal patch containing free PG only (nonlipid patch) was prepared by mixing the warm 4% agarose from above with an equal volume of PG in citrate-buffered saline (0.1 μ Ci/ml). The mixture was poured into a mold and cooled, as above.

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Example IIIDirect Drug-Release Rates: Lipid vs Nonlipid Patches

The rates of drug release from the lipid and nonlipid patches prepared in Example II were measured in a standard glass diffusion cell of the type having separable, confronting chambers. The transdermal patch being tested was placed in the upper chamber, with its free surface facing inwardly. The lower chamber, which served as a liquid reservoir for receiving drug released from the patch, was provided with opposite-side ports for flowing receptor liquid through the lower chamber. In operation, the lower cell was filled with citrate-buffered saline until the free (inwardly facing) side of the patch was completely covered with the reservoir solution. Fresh reservoir solution was flowed through the lower reservoir at about 10 ml/hr, over a 24-48 hour period. The rate of release of radioactive PG was determined by measuring the radioactivity in the 10 ml fractions collected every hour, over a 24 hour period. The samples were counted by standard scintillation counting methods.

The amount of released PG, expressed in percent total PG released per hour is plotted in Figures 3A-6A, discussed above, and the cumulative PG released, over the 24-48 hour test period, in Figures 3B-6B. The error bars on Figures 3A-6A represent standard deviation among 5-6 individual readings.

Example IVTransdermal Drug Release Rates:Liposomal versus Nonliposomal Patches

The rate of transdermal delivery of drug across full-thickness mouse skin was examined for the nonlipid and egg PC liposome transdermal patches in Example II.

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Hairless SKH-HR1 mice were supplied by Temple University Heath Science Center (Philadelphia, PA). Expenses of full-thickness skin, at least 6 cm square, were removed from the abdominal region of the animals and placed between the upper and lower chambers in the glass diffusion chamber described above, so that the skin was in contact with the entire free surface of a drug release patch placed in the upper chamber. The lower chamber was filled with the above citrate-buffered saline until the entire exposed surface of the skin expanse was in contact with the liquid. During operation, receptor liquid was flowed through the chamber at about 10 ml/hr. The 10 ml fractions collected after each hour were counted for radioactivity as above.

The levels of PG transported through the skin, expressed as percent total drug per hour are graphed in Figures 8A-10A for free PG, egg PC liposome, and DPPC liposome patches, respectively. The error bars represent standard deviation among 4 individual skin samples. Figures 8B-10B show the corresponding cumulative drug doses transported through the skin for the three patches studied.

While preferred embodiments of the invention have been described and illustrated, it will be apparent that various changes and modifications can be made without departing from the invention.

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THERE IS CLAIMED:

1. A transdermal drug release device for releasing a physiologically active, lipophilic drug to a skin surface at a selected, substantially constant release rate over a period of at least 24 hours, comprising:

an aqueous-phase matrix having a surface adapted to be placed against the skin surface, and embedded within the matrix, liposomes containing the drug in entrapped form, and composed of lipid components selected to produce such a release rate, at a liposome volume concentration that insures that a major portion of the drug is contained in the liposomes.

2. The device of claim 1, wherein the concentration of liposomes in the matrix is between about 5-25 percent by volume, and at least about 80% of the drug in the matrix is contained in the liposome.

3. The device of claim 2, wherein the drug is selected from the group consisting of nitroglycerine, prostaglandins, prostacyclines, and steroids.

4. The device of claim 2, which is effective to release drug at a relatively high release rate, wherein the liposomes lipid components are selected to include phospholipids with unsaturated acyl chain moieties.

5. The device of claim 4, wherein the liposome phospholipid components include egg phosphatidylcholine.

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6. The device of claim 2, which is effective to supply drug at a relatively low release rate, wherein the liposomes lipid components are selected to include phospholipids with predominantly saturated acyl-chain moieties.

7. The device of claim 1, wherein the matrix is a polysaccharide gel.

8. The device of claim 1, which further includes a hydrophilic drug contained in the aqueous phase of the matrix, and the lipophilic drug is adapted to increase transdermal uptake of the hydrophilic drug.

9. The device of claim 8, wherein the hydrophilic drug is selected from the group consisting of peptide hormones, water-soluble antibiotics, and anti-motion drugs.

10. The device of claim 8, which further includes a diffusion-limiting membrane interposed between the matrix and such skin surface, with the device operatively placed for transdermal drug delivery.

11. In a transdermal drug delivery system in which a physiologically active, lipophilic drug is released from an aqueous-phase matrix onto a skin surface, a method of achieving drug release from the matrix at a selected, substantially constant rate over a period of at least about 24 hours, comprising:

entrapping the drug in liposomes containing a lipid components which are selected to produce such a selected rate of drug release from the liposomes into an aqueous medium, and

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embedding the liposomes in the matrix at a concentration effective to achieve such release rate.

12. The method of claim 11, for achieving a relatively high drug release rate, wherein the lipid components are selected to include phospholipids whose acyl-chain moieties are predominantly unsaturated and/or short-chain.

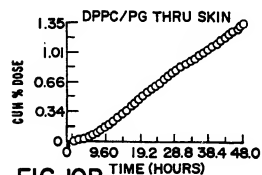
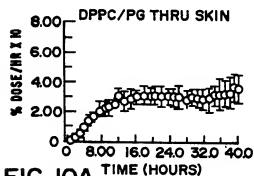
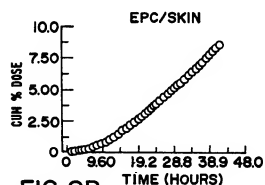
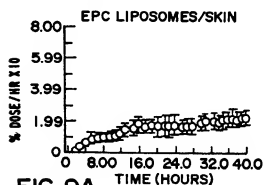
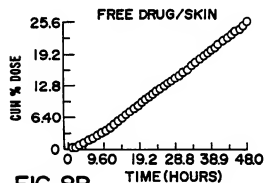
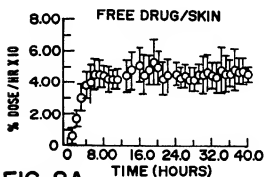
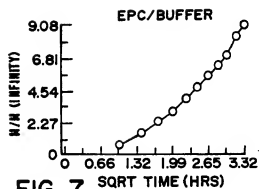
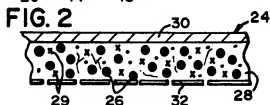
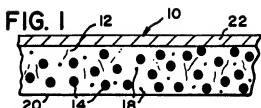
13. The method of claim 11, for achieving a relatively low drug release rate, wherein the lipid components are selected to include phospholipids whose acyl-chain moieties are predominantly saturated and/or long-chain.

14. The method of claim 11, for use in a drug delivery system containing a hydrophilic drug in the matrix, wherein the lipophilic drug is a drug transport agent effective to facilitate transdermal uptake of the hydrophilic drug.

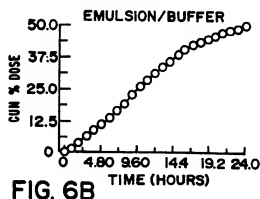
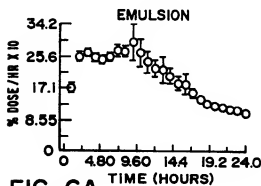
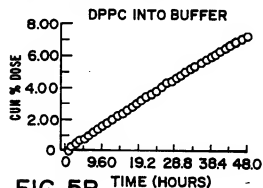
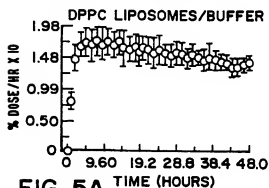
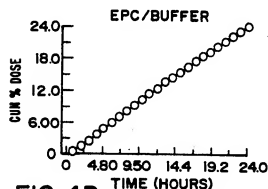
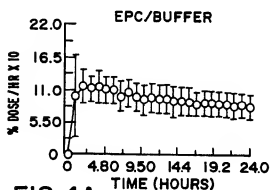
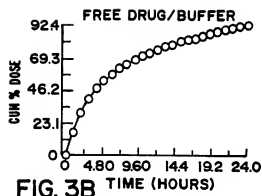
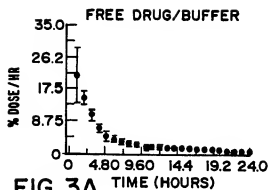
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INTERNATIONAL SEARCH REPORT

International Application No. PCT/US86/01893

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) * According to International Patent Classification (IPC) or to both National Classification and IPC INT. CL. (4) A61K 9/42; A61J 5/00; B01J 13/02; B32B 5/16 U.S. CL. 424-38; 264-4.1, 4.3, 4.6; 428-402.2																							
II. FIELDS SEARCHED <div style="text-align: center; border: 1px solid black; padding: 2px;">Minimum Documentation Searched *</div> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 20%; border: 1px solid black; padding: 2px;">Classification System</th> <th style="width: 80%; border: 1px solid black; padding: 2px;">Classification Symbols</th> </tr> <tr> <td style="border: 1px solid black; padding: 5px; vertical-align: top;">U.S.</td> <td style="border: 1px solid black; padding: 5px;">424-38 264-4.1, 4.3, 4.6 428-402.2</td> </tr> </table> <div style="text-align: center; border: 1px solid black; padding: 2px; margin-top: 5px;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched *</div>			Classification System	Classification Symbols	U.S.	424-38 264-4.1, 4.3, 4.6 428-402.2																	
Classification System	Classification Symbols																						
U.S.	424-38 264-4.1, 4.3, 4.6 428-402.2																						
III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴ <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 10%; border: 1px solid black; padding: 2px;">Category *</th> <th style="width: 70%; border: 1px solid black; padding: 2px;">Citation of Document, ¹⁵ with indication, where appropriate, of the relevant passages ¹⁷</th> <th style="width: 20%; border: 1px solid black; padding: 2px;">Relevant to Claim No. ¹⁸</th> </tr> <tr> <td style="border: 1px solid black; text-align: center; padding: 5px;">Y</td> <td style="border: 1px solid black; padding: 5px;">US, A, 3,598,123 (ZAFFARONI) 10 August 1971, see the entire document.</td> <td style="border: 1px solid black; text-align: center; padding: 5px;">ALL</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; padding: 5px;">Y</td> <td style="border: 1px solid black; padding: 5px;">US, A, 3,989,816 (RAJADHYAKSHA) 02 November 1976, see col. 1, line 60 - col. 2, line 21.</td> <td style="border: 1px solid black; text-align: center; padding: 5px;">ALL</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; padding: 5px;">Y</td> <td style="border: 1px solid black; padding: 5px;">US, A, 3,797,494 (ZAFFRONI) 19 March 1974, see the entire document.</td> <td style="border: 1px solid black; text-align: center; padding: 5px;">ALL</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; padding: 5px;">Y</td> <td style="border: 1px solid black; padding: 5px;">US, A, 4,286,592 (CHANDRASEKARAN) 01 September 1981, see the entire document.</td> <td style="border: 1px solid black; text-align: center; padding: 5px;">ALL</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; padding: 5px;">Y</td> <td style="border: 1px solid black; padding: 5px;">US, A, 4,316,893 (RAJADHYAKSHA) 23 February 1982, see col. 1, line 61- col. 2, line 29.</td> <td style="border: 1px solid black; text-align: center; padding: 5px;">ALL</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; padding: 5px;">Y</td> <td style="border: 1px solid black; padding: 5px;">Chemical Abstracts, Volume 102, No. 9, issued 04 March 1985 (Columbus, Ohio, USA), Krowczynski, "Liposomes as Drug Carriers in Transdermal Therapy," see page 355, the abstract No. 102:84351a, Pharmazie. 1984, 39(9), 627-9 (Ger).</td> <td style="border: 1px solid black; text-align: center; padding: 5px;">ALL</td> </tr> </table>			Category *	Citation of Document, ¹⁵ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸	Y	US, A, 3,598,123 (ZAFFARONI) 10 August 1971, see the entire document.	ALL	Y	US, A, 3,989,816 (RAJADHYAKSHA) 02 November 1976, see col. 1, line 60 - col. 2, line 21.	ALL	Y	US, A, 3,797,494 (ZAFFRONI) 19 March 1974, see the entire document.	ALL	Y	US, A, 4,286,592 (CHANDRASEKARAN) 01 September 1981, see the entire document.	ALL	Y	US, A, 4,316,893 (RAJADHYAKSHA) 23 February 1982, see col. 1, line 61- col. 2, line 29.	ALL	Y	Chemical Abstracts, Volume 102, No. 9, issued 04 March 1985 (Columbus, Ohio, USA), Krowczynski, "Liposomes as Drug Carriers in Transdermal Therapy," see page 355, the abstract No. 102:84351a, Pharmazie. 1984, 39(9), 627-9 (Ger).	ALL
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<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>* Special categories of cited documents: ¹⁶</p> <p>"A" document defining the general state of the art</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document cited for special reason other than those referred to in the other categories</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> </div> <div style="width: 50%;"> <p>"P" document published prior to the international filing date but on or after the priority date claimed</p> <p>"T" later document published on or after the international filing date or priority date and not in conflict with the application, but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance</p> </div> </div>																							
IV. CERTIFICATION <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; border: 1px solid black; padding: 5px; vertical-align: top;"> Date of the Actual Completion of the International Search * <div style="text-align: center; font-weight: bold;">20 November 1986</div> International Searching Authority * <div style="text-align: center;">ISA/US</div> </td> <td style="width: 50%; border: 1px solid black; padding: 5px; vertical-align: top;"> Date of Mailing of this International Search Report * <div style="text-align: center; font-weight: bold; font-size: 1.2em;">03 DEC 1986</div> Signature of Authorized Officer ²⁰ <i>John M. Kilcoyne</i> John M. Kilcoyne </td> </tr> </table>			Date of the Actual Completion of the International Search * <div style="text-align: center; font-weight: bold;">20 November 1986</div> International Searching Authority * <div style="text-align: center;">ISA/US</div>	Date of Mailing of this International Search Report * <div style="text-align: center; font-weight: bold; font-size: 1.2em;">03 DEC 1986</div> Signature of Authorized Officer ²⁰ <i>John M. Kilcoyne</i> John M. Kilcoyne																			
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FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

P, Y

Chemical Abstracts, Volume 104, No. 3,
issued 20 January 1986 (Columbus, Ohio,
USA,) Komatsu, "Preparation of Drug-
Liposome Inclusion Compounds", see page
300, the abstract No. 104:24197m, Jpn.
Kokai Tokkyo Koho JP 60,152,410 [85,
152,410] 10 August 1985.

ALL

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹⁰

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers because they relate to subject matter ¹¹ not required to be searched by this Authority, namely:

2. ☐ Claim numbers because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out ¹², specifically:

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ¹³

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
☐ No protest accompanied the payment of additional search fees.

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 9/127, 7/00	A1	(11) International Publication Number: WO 95/35095 (43) International Publication Date: 28 December 1995 (28.12.95)
(21) International Application Number: PCT/EP95/02397 (22) International Filing Date: 21 June 1995 (21.06.95) (30) Priority Data: 08/264,204 22 June 1994 (22.06.94) US (71) Applicant (for all designated States except US): YISSUM RESEARCH DEVELOPMENT COMPANY OF THE HEBREW UNIVERSITY OF JERUSALEM [IL/IL]; 46 Jabotinsky Street, P.O. Box 4279, 91042 Jerusalem (IL). (71)(72) Applicant and Inventor: TOUITOU, Elka [FR/IL]; 6 Demunit Street, Givat Canada, 93890 Jerusalem (IL). (74) Agent: KRAUS, Walter; Kraus, Weisert & Partner, Thomas-Wimmer-Ring 15, D-80539 Munich (DE).	(81) Designated States: AU, BR, CA, CZ, FI, HU, JP, KR, LK, MX, NO, NZ, PL, RO, RU, SK, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>	
(54) Title: COMPOSITIONS FOR APPLYING ACTIVE SUBSTANCES TO OR THROUGH THE SKIN (57) Abstract A cosmetic or medical composition of topical application to the skin. It results in the transdermal passage of an active ingredient, or in the introduction of such agent into the skin. The essential components of such compositions are a phospholipid, a lower aliphatic alcohol of two to four carbon atoms, optionally with propylene glycol, water and a compatible active ingredient. The alcohol content is generally from 20 to 50 %, and when glycol is present, the combined percentage of alcohol and glycol being up to about 70 %. The compositions are suitable for the topical application of a wide variety of cosmetic and pharmaceutical compounds.		

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DESCRIPTIONCOMPOSITIONS FOR APPLYING ACTIVE SUBSTANCES TO OR THROUGH THE SKINFIELD OF THE INVENTION:

The invention relates to novel compositions containing phospholipids, short chain alcohols (C2-C4) and water. These compositions may also contain polyols.

Preferred compositions contain phospholipid, ethanol (EtOH), water (DDW), and propylene glycol (PG).

This invention relates to pharmaceutical, cosmetic, veterinary or phytopharmaceutical compositions for delivery to skin, membranes, or tissues, which enhance the delivery of the active agent at the site of application, where the agent may form a reservoir in the skin (membrane, tissue) or may be absorbed systemically into the blood circulation.

The compositions are hydro-alcoholic or hydro/alcoholic/glycolic phospholipid systems in which the concentration of alcohols, glycols, or their combination is relatively high. The main components of these systems are: phospholipids [with various chemical structures like phosphatidylcholine (PC), hydrogenated PC, phosphatidic acid (PA), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylglycerol (PPG), phosphatidylinositol (PI), hydrogenated PC and others], ethanol (or other short chain alcohols), water and propylene glycol (or other glycols).

The novel composition enables the delivery of higher concentrations of active agent to/through membrane (skin). The delivery can be modulated by changes in alcohol:water or alcohol-polyol:water ratio.

State of the Prior Art

In drug delivery, there are a number of enhancing agents, some used in research and some on the market, for promoting and increasing the amount of drug delivered to/through the skin. Examples of these enhancing agents are: DMSO, pyrrolidone derivatives, n-decylMSO, some surfactants, oleic acid, ethanol, Azone[®], and others (Barry, 1989).

Phospholipids are known for their broad use in liposomal systems as well as emulsifiers in the preparation of emulsions. All these systems used for pharmaceutical or cosmetic purposes are aqueous systems

with small if any concentration of alcohol and/or glycol for preservation and/or improving texture of the formulation. Emulsions containing phospholipids are prepared by mixing an aqueous and an oily phase, in some cases followed by use of an homogenizer.

Preparation of liposomal systems involves the use of organic solvents such as chloroform, alcohols and others. The prior art teaches away from high concentrations of alcohol in the final liposomal preparations. In some methods of preparation, an organic phospholipid solution is evaporated to form a lipidic film, which is then hydrated to give an aqueous vesicular system (Riaz et al., 1988). In alternative methods, liposomes are prepared by injecting an ethanolic solution of lipid into an aqueous solution, resulting in a dilute ethanolic solution (2.5-7.5% ethanol) (Batzri et al., 1973) or by dilution of proliposomes (Leigh, 1991). The alcohol, is then removed by different means such as dialysis (Kremer et al, 1977) or is diluted. The alcohol, if present is in low concentrations only, less than about 20% in the final product (e.g. 7.5%, Kremer et al, 1977; Leigh, 1991).

Brief Description of the Invention

The invention relates to compositions which are hydro/alcoholic or hydro/alcoholic/glycolic phospholipid systems in which the concentration of alcohol or its combination with polyol is relatively high. We call these compositions: Ethosomal systems. The systems described in this invention are pharmaceutical, cosmetic, phytopharmaceutical or veterinary compositions for application to the skin (or other tissues) comprised of combinations of phospholipids, alcohols, water and glycol (polyols), as major components. An ethosomal system is a skin permeation enhancing system which has the potential to form ethosomes, which are "soft" vesicles formed from phospholipid in the presence of water and ethanol (alcohol) and sometimes glycols (polyols). The size of the vesicles depends on the water: alcohol ratio (see Tables 3-5) and on the phospholipid concentration. On the skin, the ethosomes change their size by fusing together as a result of the change in solvent ratio. In the preparation, the vesicle size does not change since the ratio between the solvents is constant. Penetration and evaporation of the alcohol following application to the skin allows the transition from small to large vesicles, which grow in size until a film is formed.

An important characteristic of ethosomes is enhanced membrane permeability for various compounds. Ethosomal systems, vesicular in

nature, depending on the ratio of the components and the chemical structure of the phospholipids, can be comprised of very small entities (nm's) up to larger vesicles (mm's) (see Tables.3-5). High alcoholic (organic solvent) concentration favors the production of ethosomes in nm's range while high aqueous and phospholipid concentrations favor the formation of large size ethosomes. As examples, formulation 509 (Table 4) containing 60% organic solvent and 38% water has a mean population of tens of nm's, while formulation 510 containing 50% organic solvent and 48% water has a mean population of 1mm. In system 509 the concentration of ethanol was 48% while in formulation 510 the ethanol concentration is only 20%, showing that the alcohol concentration is of great importance in determining vesicle size. The phospholipids which can be used are: phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidic acid (PA), phosphatidylethanolamine (PE), phosphatidylglycol (PPG), hydrogenated PC and others. Some preferred phospholipids are soya phospholipids such as Phospholipon 90 (PL-90). The concentration of phospholipid ranges between about 0.5-10% w/w. Cholesterol at concentrations ranging between about 0.1-1% can also be added to the preparation. Examples of alcohols which can be used are: ethanol and isopropyl alcohol. Examples of glycols are propylene glycol and Transcutol^R. The source of the phospholipids can be egg, soybean, semi-synthetics, and synthetics. Non ionic surfactants can be combined with the phospholipids in these preparations e.g. PEG-alkyl ethers (Brij-52). Cationic lipids like cocoamide, Pae alkyl amines, dodecylamine, cetriride, and like. The concentration of alcohol (EtOH etc.) in the final product ranges from about 20-50%. The concentration of the non-aqueous phase (alcohol and glycol combination) may range between about 22 to 70%. The rest of the carrier contains water and possible additives. Vesicle formation is dependent on the water: alcohol ratio. This ratio is kept constant in the product, therefore, no changes in the entities population occur. Nevertheless, penetration and evaporation of the alcohol following application to the skin allows the transition from small vesicles to a larger ones, finally resulting in film formation. In contrast to the present state of the art where "tough" liposomes accomplished by addition of different substances like cholesterol to the phospholipids and in absence of alcohol, this invention relates to "soft" vesicles, that can be easily formed in a hydroalcoholic medium. One of the important properties of these systems is that small entities can penetrate into the skin, while larger vesicles can form a reservoir

in the skin and a film on the skin surface as a result of solvent evaporation taking place after the application.

These carriers can be used to deliver various active agents such as: peptides, anti-aging, tanning agents, , vitamins, antiviral drugs, psoriasis treatment agents, hormones, medicinally active componenets of plants such as oleoresins, volatile oils, glycosides, alkaloides, terpenes and others.

Examples of other active agents which can be used with these systems are as follows: drugs like nicotine, nitroglycerine, estradiol (or like), testosterone (or like), progesterone, nifedipine, minoxidil, tetrahydrocannabinol (THC) or other cannabinoids, xanthines, anxiolytics (diazepam and others), antiepileptic (valnoctamide and others), diclofenac (and other NSAIDs), antibiotics, corticosteroids, tocopherol, 5-FU, acyclovir, colchicine, prazosin, papaverine, miconazole nitrate, ketoconazole and other antifungals, enzymes like SOD; peptides and amino acids.

Methods

In vitro skin penetration/permeation measurements

Skin permeation experiments were carried out in our laboratory as previously described (Touitou, 1986; Touitou and Fabin, 1988).

Full thickness skin

The animals used in these investigations were hairless mice obtained from the Weizmann Institute, Rehovot, Israel. The skin of 5-7 week old male mice was excised before the experiment, examined for integrity and cut for diffusion cell assembly. In most of the experiments, the skin from the abdominal site was used.

In vitro permeation experiments in horizontal diffusion cells.

The Valia-Chien cell assembly was used to perform the experiments on permeation of the drugs from various donor compositions through hairless mouse skin and the concentration in the skin. Mouse skin treated as described above was mounted in cells with a surface area of 0.64 cm^2 and half-cell volume of 3 ml. The receiver compartments contained a medium (hydroalcoholic, water, or buffer solutions) for ensuring pseudo-sink conditions. During the experiment, samples were withdrawn and mixed with scintillation cocktail (Packard, USA) and assayed in the Kontron Betamatic Scintillation Counter (Lumitron Scientific Industries) or directly assayed by HPLC. The sample volumes were replaced with fresh solution. The results were treated using the "Transderm" computer program (Touitou and Fabin, 1988). Each experiment was tetraplicated. The two-tailed, paired Wilcoxon test was used for determining the statistical significance of the effect of enhancers on the permeability coefficient changes. For these analyses, the "Balance" (IBM) computer program was used.

Skin Permeation Determination in Franz Cells

The permeation flux of drugs and the quantity of drug in the skin from the test system was measured in vertical cells (Franz diffusion cells). The experimental system conditions were: receiver temperature 37°C and receiver volume 4-8 ml. The samples were assayed either by scintillation counting or by HPLC. The results are presented as a mean of 4 experiments and were analyzed by Transderm and by Balance (IBM) computer programs.

Size Distribution of Liposomes:

The size distribution of ethosomes was measured in a Malvern autosizer (Malvern). (See Tables 3-5).

Method of Preparation and Compositions:

Examples of compositions are given in Tables 1 and 2.

a) Ethosomal system containing a relatively high concentration of Ethanol or Ethanol and Propylene Glycol was obtained as follows:

A lipophilic drug(10%) and PL-90(5%) were dissolved in an EtOH-PG(62.4%) mixture at room temperature. DDW(22.6%) was then added with vigorous stirring.

See example II-Immune agent.

b) Ethosomal systems containing a relatively high concentration of Ethanol (20-50%) or Ethanol and Propylene Glycol (22-70%) were obtained as follows:

PL-90 (0.5-10%) was dissolved in the EtOH-PG mixture. Hydrophilic drugs were dissolved in DDW and added to the PL-90 solution.

c) Ethosomal system was prepared by mixing (Heidolph mixer) PL-90 and water in concentrations as in "b" and heating to dispersion at 60-70°C. The dispersion was then cooled (ice bath) with constant mixing for 30 minutes. To the above dispersion a solution of 2% Minoxidil in ethanol-propylene glycol (concentration as above) was added with vigorous mixing. A vesicular system was obtained. The preparation may be passed through a homogenizer (like Gaoline).

d) Minoxidil ethosomes were prepared by gently heating or at room temperature, a solution of soybean lecithin (Phospholipon 90) and Minoxidil in a propylene glycol ethanol mixture. Distilled water or buffer solution was added to the above system. A vesicular system was formed. The preparation may be passed through a homogenizer (like Gaoline). concentrations of ingredients as in "c".

e) A vesicular system containing a relatively high concentration of Ethanol or Ethanol and Propylene Glycol was obtained as follows: A dispersion containing soya phospholipid (Phospholipon 90), Minoxidil, Ethanol, propylene glycol, double distilled water or buffer solution, is passed through a homogenizer in order to reduce particle size. concentrations of ingredients as in "c".

f) Composition and preparation as described in a-c but containing 0.2 % cholesterol in addition.

g) As in a-d where mixtures of phospholipids are used.

h) The vesicular systems can be incorporated in various carriers such as: PVP/VA (gels, membranes, solutions), PVP (gels, membranes, solutions) carbomer gels, polaxomer (gels, solutions), emulsions, adhesives, creams, Pluronic F127 or Tetronic gels and the like, cellulose derivatives gels, plant extract gels (aloe vera gel etc), and the like.

Table 1: Examples of skin permeation enhancing systems containing various drugs
Systems
 DYPH1 DYPH2 MND200 SOD Immune THC 1 MM33 MM39 MM42 MM43

COMPONENTS	1%	1%	7µlCl/ml	6%	10%	2%	2%	2%	5%	0.1%
DYPHYLLINE										
ACYCLOVIR										
DICLOFENAC										
SOD										
TOQUINIMEX										
MINOXIDIL										
THC										
PL-90	5%	5%	2%	2%	5%	2%	2%	2%	5%	5%
CHOLESTEROL	-	-	-	-	-	-	-	-	-	-
PG	10.8%	-	19.6%	18.4%	31.2%	19.6%	19.2%	19.2%	19.5%	18.6%
DDW	47%	47%	49%	46%	22.6%	29.4	28.8%	48%	48.8%	27.9%
EtOH	28.2%	47%	29.4%	27.6%	31.2%	49%	48%	28.8%	29.3%	46.5%

Examples of compositions and preparation methods:

Example I- SOD ethosomal preparation

A)	Amerchol L-101	57.1%	4 parts
	Amerlate P	14.3%	1 part
	Brij 52	28.6%	2 parts
B)	Carbopol 934P	1%	
	DDW	98%	
	TEA	1%	
	SOD	6%	
C1)	PL-90	2%	
	DDW	46%	
C2)	EtOH 3	27.6%	
	PG	18.4%	

Gel preparation:

1. Preparation of "A" by melting the ingredients in a water bath. Cool while stirring.
2. Disperse Carb 934 P in DDW at room temperature.
3. Mix A and B in the ratio of 7% of A with 93% B and add TEA.

System preparation:

4. SOD was dissolved in a PL-90 dispersion in DDW [obtained by heating in a water bath of 70°C with constant mixing (C1)].
5. An EtOH:PG mixture (C2) was prepared and added to C1 with vigorous mixing and cooling in an ice bath.

Final formulation:

6. Mix gel with the system in a 1:1 ratio.
- | | |
|------------------|-------|
| Drug | 3% |
| Lipid phase (A) | 3.5% |
| Gel (B) | 46.5% |
| PL-90 + DDW (C1) | 22% |
| PG + EtOH(C2) | 25% |

Example II- IMMUNE AGENT (ROQUINIMEX) ethosomal preparation

A) Pluronic F127	40%
DDW	60%
B) ROQUINIMEX	10%
PL-90	5%
EtOH 95%	31.2%
PG	31.2%
DDW	22.6%

Prepare a Pluronic gel of 40% in DDW. Dissolve Roquinimex and PL-90 in the EtOH-PG mixture. Add DDW to the latter with vigorous stirring.

Add B to A with vigorous stirring, at a ratio of 1:1.

Example III- THC Ethosomal preparation

THC	1%
PL-90	4.2%
EtOH	51.7%
PG	15%
PVP-VA S-630	12.5%
DDW	16.6%

THC and PL-90 were dissolved in an EtOH-PG mixture with gentle heating while mixing. DDW was added with continuous stirring. PVP-VA was slowly added while stirring. The preparation was sonicated in 3 cycles of 5 minutes with 5 minutes rest between each cycle.

Example IV- Minoxidil ethosomal preparation (MM50-G)

A) Carbopol 934P	0.75%
Ethomeen C/15	0.75%
DDW	73.5%
EtOH	25%
B) Minoxidil	4%
PL-90	5%
EtOH 95%	40%
PG	20%
DDW	31%
Tocopherol	0.02%

MM50-G, Final concentrations:

Carbopol 934 P	0.38%
Ethomeen C/15	0.38%
Minoxidil	2%
PL-90	2.5%
EtOH (95%)	32.5%
PG	10%
DDW	52.3%

Carbopol gel was prepared by dispersing Carbopol 934P in DDW and Ethomeen was added. Ethanol was added to obtain an hydroalcoholic gel. The drug, antioxidant, and PL-90 were dissolved in the EtOH -PG mixture with gentle heating (30°C). DDW was added with vigorous stirring to obtain the system.

The system may be homogenized.

B was added to A at a 1:1 ratio.

The mean size of vesicles was found to be 35nm

Example V- Minoxidil ethosomal preparation (MM)

As in example IV (MM50-G) with the following variations:

- The gel:system ratio may be changed to 1:2, 1:3, or 1:4.
- The ratio between solvents can be changed: EtOH (25-49%), PG (0-20%), and DDW (25-49%) can be changed.
- The PL-90 concentration can be raised to about 10%.

Example VI- DICLOFENAC SODIUM ethosomal preparation

Diclofenac sod.	1%
PL-90	1%
Carbopol 934	0.9%
Ammonium 10% soln.	1.8%
EtOH	21.9%
DDW	68.9%
PG	4.16%

Example VII- ACYCLOVIR ethosomal preparation

Acyclovir	5%
Ammonium 10% soln.	1.66%
Carbopol 934	0.83%
PL-90	2.5%
DDW	58.3%
EtOH	27.5%
PG	4.16%

Table 2: Other Examples

Sys. No.	Ingredients, % w/w					Method*
	PL-90	DDW	EtOH	PG	Other	
500	2	48	30	20		Hot
501	2	48	30	20		Cold
502	2	30	48	20		Hot
503	2	30	48	20		Cold
504	5	45	30	20		Hot
505	5	45	30	20		Cold
506	5	30	45	20		Hot
507	5	30	45	20		Cold
508	2	30	38	30		Cold
509	2	38	30	30		Cold
510	2	48	20	30		Cold
511	1.8	30	38	30	0.2(M(-)*)	Cold
512	5	30	45	20		Cold
513	5	35	30	30		Cold
514	2	33	30	30	5(Brij52)	Cold
515	17	26	40	17		Cold
516	9.3	44.4	27.7	18.5		Cold
517	5	20	40	40	5(Brij52)	Cold
518	2	47	30	20	1(Col**)	Cold
525	10	64	26	0		Hot
529	1.7	55.4	34.3	8.6		Cold

PL-90: phospholipid; DDW: water; EtOH: ethanol; PG: propylene glycol

*M(-): l-Menthol

**Col: Colchicine

* See "Hot" or "Cold" methods described below.

"Cold" method:

Phospholipid is dissolved in ethanol at room temperature by vigorous stirring with the use of Heidolph mixer. Propylene glycol is added during the stirring. The mixture is heated to 30°C in a water bath. The water heated to 30°C in a separate vessel is added to the mixture which is then stirred for 5 minutes in a covered vessel.

"Hot" method:

The phospholipid is dispersed in water by heating in a water bath at 40°C until a colloidal solution is obtained. In a separate vessel ethanol and propylene glycol are mixed and heated to 40°C. Once both mixtures reach 40°C the organic phase is added to the aqueous one.

During the preparation process the drug is dissolved in water or in ethanol depending on its hydrophilic/hydrophobic properties.

EXAMPLES OF SKIN PERMEATION PARAMETERS OF VARIOUS DRUGS FROM THESE SYSTEMS (Kp= permeability coefficient; Qs = quantity of drug in the skin at the end of the experiment)

Kp reflects the permeation through the skin and Qs reflects the reservoir formation in the skin.

Example VIII

MM33= MM36	vs.	blank MM (Minoxitrim ^R , Trima, Israel)
(see Table 1)		(2% minoxidil)

Kp= $1.36 \times 10^{-2} \text{ cm} \cdot \text{hr}^{-1}$	$8.84 \times 10^{-4} \text{ cm} \cdot \text{hr}^{-1}$
---	---

Qskin= 0.658 mg/cm^2	0.0479 mg/cm^2
--------------------------------	--------------------------

The ethosomal system showed an increase of 15.4 times in Kp and 13.7 times in Qs.

Example IX

MM39 (see Table 1)	MM blank(as above)
--------------------	--------------------

Kp= $1.96 \times 10^{-3} \text{ cm} \cdot \text{hr}^{-1}$	$5.75 \times 10^{-5} \text{ cm} \cdot \text{hr}^{-1}$
---	---

Qskin = 0.138 mg/cm^2	0.0378 mg/cm^2
---------------------------------	--------------------------

The ethosomal system showed an increase of 34 times in Kp and 3.7 times in Qs.

Example X

THC (as in ex. III)

THC in PG:EtOH (1:1)

 $K_p = 7.2 \times 10^{-3} \text{ cm}^2 \cdot \text{hr}^{-1}$ $2.03 \times 10^{-3} \text{ cm}^2 \cdot \text{hr}^{-1}$

The ethosomal system showed an increase of 3.5 times in K_p . No significant changes in other kinetic parameters were observed

Example XI

MM50G (as in ex. IV)

MM blank

$Q_{\text{receiver}} = 0.135 \mu\text{g}/\text{cm}^2$
(24 hours)

 $0.023 \mu\text{g}/\text{cm}^2$

The ethosomal system showed an increase of 5.9 times in K_p

Example XII

ACV11 (as in ex VII)

Zovirax^R

Q_{receiver}
(6 hours)

 $2.9 \pm 1.57 \mu\text{g}/\text{cm}^2$ $3.065 \pm 0.38 \mu\text{g}/\text{cm}^2$ Q_{skin} $81.35 \pm 5.58 \mu\text{g}/\text{cm}^2$ $18.35 \pm 8.56 \mu\text{g}/\text{cm}^2$

The ethosomal system showed no significant change in K_p and an increase of 4.4 times in Q_s .

Example XIII

MM122

MM122 blank a

MM122 blank b

2%PL-90, 2% Minoxidil

2%PL-90, 2% Minoxidil

2% Minoxidil

48% EtOH 95, 28% DDW,

in EtOH

in EtOH

20% PG

$Q_{\text{rec}} = 3672 \pm 378 \mu\text{g}/\text{cm}^2$
(24 hours)

 $217.5 \pm 118.5 \mu\text{g}/\text{cm}^2$ $66.9 \pm 22.1 \mu\text{g}/\text{cm}^2$ $Q_{\text{skin}} = 570 \pm 130.5 \mu\text{g}/\text{cm}^2$ $139.1 \pm 25.3 \mu\text{g}/\text{cm}^2$ $53.5 \pm 12 \mu\text{g}/\text{cm}^2$

The ethosomal system showed an increase of 17 times in K_p and 4.1 times in Q_s relative to a 2% phospholipid solution in ethanol (blank a) and an increase of 55 times in K_p and 11 times in Q_s relative to an ethanolic solution (blank b). These results clearly indicate that the ethosomal system is a much more effective penetration enhancing system than ethanol or an ethanolic solution of phospholipid.

Example XIV

	MND200	Blank MND (only solvents)
Kp =	$4.27 \times 10^{-3} \text{ cm} \cdot \text{hr}^{-1}$	$1.57 \times 10^{-3} \text{ cm} \cdot \text{hr}^{-1}$
Q _{skin} =	$2.75 \times 10^{-5} \text{ mg/cm}^2$	$2.35 \times 10^{-5} \text{ mg/cm}^2$
lag time =	0:41 hrs	2:30 hrs

The ethosomal system showed an increase of 2.7 times in Kp no significant change in Qs and a decrease of 5 times in lag time.

Example XV

Differential Scanning Calorimetric (DSC) data on a system containing 5% PL-90 in water and an ethosomal system No. 525 (see Table 2 for exact composition) are presented in Figs.1 and 2, respectively. The thermograms clearly show a decrease in T_m (transition temperature) value for the ethosomal system which indicates an increase in phospholipid vesicle's fluidity.

Example XVI:

Figures 3 and 4 present photographs of vesicles in ethosomal systems 510 and 529 (Table 2) as seen by means of a computerized image analyser connected to a light microscope (Cue 2 Galai- Axioscope Zeiss). These photos clearly show the presence of vesicles (ethosomes) in systems containing 20 and 34.3% ethanol, respectively.

Example XVII

There were prepared: Caffeine ethosomes (liposomal systems containing 20.9 and 35% ethanol) versus Caffeine liposomes with 5% ethanol.

All three preparations contain the same concentration of caffeine (3%) and the same phospholipid (Phospholipon 90) at 5% concentration, without propylene glycol.

Methods

In vitro skin penetration/permeation measurements.

Skin permeation experiment was carried out in our laboratory by using the Valia-Chien cell assembly to perform the experiment.

The skin of 5-7 week old male mice was used in this investigation (nude mice obtained from the Weizmann Institute, Rehovot, Israel.). The skin was examined for integrity and cut for mounting on diffusion cell assembly. The skin from the back site was used.

The skin was mounted in cells with a surface area of 0.64 cm² and half-cell volume of 3 ml. The receiver compartments contained water for ensuring pseudo-sink conditions. Samples were directly assayed by HPLC (Touitou et al, 1994). The experiment was run for 24 hours. Each formulation was tested in three cells.

Caffeine Ethosomes

A) Caffeine	3.0%
Sod. Salicylate	4.8%
Distilled Water	52.2%

B) Phospholipon-90	5.0%
Ethanol	35.0%

Example XVIII

A composition was prepared as set out on page 6, b.

A) Dissolve Caffeine and Sod. Salicylate in the water.

B) Dissolve the phospholipid in ethanol. Add A to B with vigorous stirring.

Caffeine Liposomes

Caffeine	3.0%
Sod. Salicylate	4.8%
Phospholipon-90	5.0%
Ethanol	5%
Distilled Water	82.2%

Preparation as above.

Results

Q_{rec} = quantity of drug in the receiver compartment of the diffusion cell at the end of the experiment.

Q_{rec}

Caffeine Liposomes (5% ethanol)	Caffeine Ethosomes (20.9% ethanol)	Caffeine Ethosomes (35% ethanol)
86.8±17.1 $\mu\text{g}/\text{cm}^2$	496.6±71 $\mu\text{g}/\text{cm}^2$	4794.3±812 $\mu\text{g}/\text{cm}^2$

These results clearly indicate that the ethosomal system according to the invention containing 35% ethanol enabled an enhanced delivery of caffeine through the skin of 53 times higher than the liposomes containing caffeine with 5% ethanol. The above proves the outstanding improvement resulting from a high content of alcohol in the liposomal system, in the presence of liposomes.

An increase of skin penetration from $87 \mu\text{g}/\text{cm}^2$ to about $4794 \mu\text{g}/\text{cm}^2$, i.e., an increase by a factor of about 53 times as large, demonstrates a dramatic and unexpected result of the novel liposomes with a high ethanol content, termed "ethosomes". A 53-fold skin penetration could not be expected at all on the basis of the prior art, which clearly teaches away from the present invention, i.e. that a high ethanol content is detrimental for liposomal preparations, and that the ethanol content of the final liposome preparation ought to be reduced so as to remove a large part of the initial ethanol content or by dilution.

Example XIX

Further experiments were carried out with Minoxidil, comparing liposomes with a high ethanol content with Minoxidil in the vehicle.

The experiments were carried out as follows:

The formulations tested were: Minoxidil ethosomes (liposomal systems containing ethanol) versus Minoxidil in vehicle. Both preparations contain the same concentration of Minoxidil (1%).

Methods

In vitro skin permeation measurements:

Skin permeation experiment was carried out in our laboratory by using the Franz cell assembly to perform the experiment.

Frozen back side skin of a 5-7 week old male mice was used in this investigation (nude mice obtained from the Weizmann Institute, Rehovot, Israel). The skin was examined for integrity and cut for mounting on diffusion cell assembly.

Nude mouse skin was mounted in cells with a surface area of 1.77 cm^2 and receiver volume of about 7 ml. The receiver compartments contained 1/150M pH7 phosphate buffer for ensuring pseudo sink conditions. Samples were directly assayed by HPLC. The experiment was run for 12 hours. Each formulation was tested in three cells.

Minoxidil Ethosomes

Minoxidil	1%
Phospholipon	2%
Ethanol 95%	40%
Distilled Water	57%

Minoxidil vehicle

Minoxidil	1%
Ethanol 95%	40%
Distilled Water	59%

Results

Q_{rec} = quantity in the receiver compartment of the diffusion cell at the end of experiment.

	Q_{rec}
Minoxidil vehicle	Minoxidil ethosomes
$16.26 \pm 2.8 \text{ } \mu\text{g}/\text{cm}^2$	$64.02 \pm 22.5 \text{ } \mu\text{g}/\text{cm}^2$

The above results, of about $16 \mu\text{g}/\text{cm}^2$ versus about $64 \mu\text{g}/\text{cm}^2$ skin penetration of the two preparations, demonstrates that the "ethosomes" of the invention resulted in an about 4-fold skin penetration compared with the penetration of the active substance in the vehicle only, i.e. not in liposome form. These ethosomes were without propylene glycol.

Example XX

The following experimental results, relate to various liposome systems of the invention containing 1% sodium diclofenac as model drug and in which various compositional factors have been changed: 1. the concentration of alcohol 2. the phospholipid 3. the type of alcohol.

The results demonstrate: 1. the cruciality of high concentrations of alcohol, and that the high skin permeation from ethosomal systems of the invention is still obtained: 2. with an additional example of phospholipid (Lipoid E 75-containing phosphatidyl ethanolamine and phosphatidyl choline isolated from egg, produced by Lipoid KG; Germany, 3. with isopropyl alcohol.

Methods:

In vitro skin permeation measurements.

Skin permeation experiment was carried out in our laboratory by using the Valia Chien assembly to perform the experiments.

Frozen back side skin of a 5-7 week old mouse was used in these experiments (nude mice obtained from the Weizman Institute, Rehovot, Israel). The skin was examined for integrity and cut for mounting on diffusion cell assembly.

The skin was mounted in cells with a surface area of 0.64 cm² and receiver volume of about 3 ml. The receiver compartments contained 1/150M pH7 phosphate buffer for ensuring pseudo sink conditions.

Samples were directly assayed by HPLC. The experiments were run for 17 hours. Each formulation was tested in triplicates.

FORMULATIONS:

	XA	XB ₁	XB ₂	XC	XD	XE
	<u>% w/w</u>					
Sod.Diclofenac	1	1	1	1	1	1
Phospholipon 90	5	5	5	-	-	5
Lipoid E	-	-	-	5	5	-
Ethanol	5	21	35	5	35	-
Isopropyl Alcohol	-	-	-	-	-	35
Distilled Water	89	73	59	89	59	59

Results:

Q_{rec} = quantity in the receiver compartment of the diffusion cell at the end of experiment.

$Q_{rec}, \mu\text{g}/\text{cm}^2$					
XA	XB ₁	XB ₂	XC	XD	XE
37.7±11	144.1±14	309.2±29	57.6±25	285.2±37	1558.8±778

These results clearly indicate that:

1. The ethosomal systems of the hydrophilic drug diclofenac sodium, containing a high concentration of ethanol (35%), give an

enhanced delivery of drug through the skin of eight times higher than the preparation containing only 5% ethanol;

2. The enhancing effect is also obtained with isopropyl alcohol;
3. The enhancing effect is obtained when the composition of phospholipids is changed;

The above results demonstrate the improvement in permeation with novel liposomal ("Ethosomal") systems of the invention.

Remark: all these systems are without propylene glycol (PG) showing the noncriticality of PG.

The invention is illustrated with reference to the enclosed Figures and photos, in which:

Fig. 1 is a graph showing Differential Scanning Calorimetric data of an ethosomal system containing 5% PL-90 in water.

Fig. 2 is a graph showing Differential Scanning Calorimetric data of ethosomal system No. 525 (See Table 2).

Fig. 3 is a photograph of ethosomal system No. 510 (See Table 2).

Fig. 4 is a photograph of ethosomal system No. 529 (See Table 2).

TABLE No. 3

MALVERN AUTOSIZER II c for elthosomal system No. 501 (see Table 2)

Sample: 501; Vortex t = 22.6; Aperture: 200

Data from accumulation file sum of 5 blocks

Temperature: 22.6°C; Viscosity: 4.1800; Refractive Index: 1.365;

Angle: 90.0

Particle size distribution (nm.) Graph of Distribution of mass

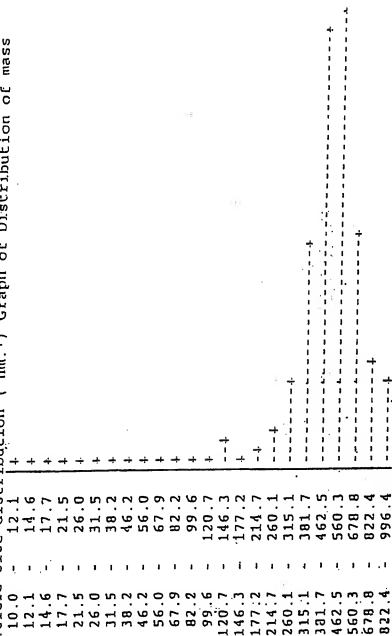


TABLE No. 4

MALVERN AUTOSIZER II c for collosomal system No. 509 (see Table 2)

Sample: 509; Vortex + US; Non diluted

Data from accumulation file sum of 8 blocks

Temperature: 22.6°C; Viscosity: 4.8200; Refractive Index: 1.376;

Angle: 90.0

Particle size distribution (nm.) Graph of Distribution of mass

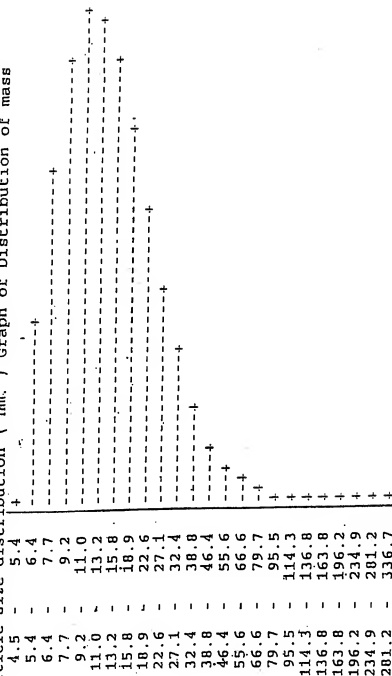


TABLE No. 5

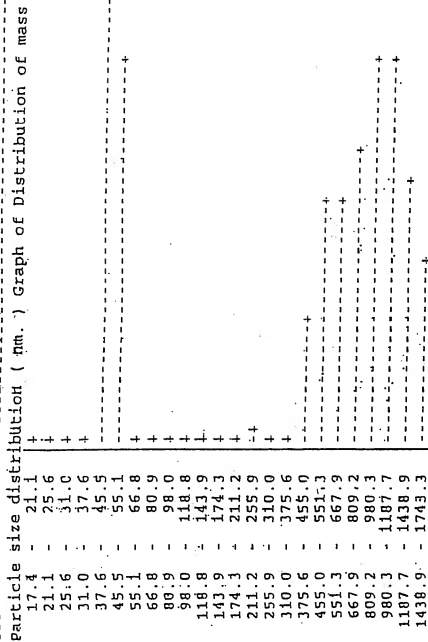
MALVERN AUTOSIZER II c for ethosomal system No. 510 (see Table 2)

Sample: 510; Vortex + US; Non diluted

Data from accumulation file sum of 6 blocks

Temperature: 22.6°C; Viscosity: 4.6400; Refractive Index: 1.374;

Angle: 90.0



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CLAIMS:

1. A liposomal composition for medical or cosmetic use, for topical application to the skin, resulting in the enhanced transdermal passage or introduction of an active ingredient into the skin, which composition contains vesicles in the size range from the nm range to the μm range, which composition comprises from 0.5% to 10% phospholipids, from 20% to 50% of a C_2 to C_4 alcohol, from 0 to 30% glycol, at least 20% water and at least one active ingredient where the combined alcohol and glycol content does not exceed 70%.
2. A composition according to claim 1, where the penetration through the skin is at least four times that of a similar liposome composition containing less than 5% alcohol.
3. A composition according to any of claims 1 or 2, where the phospholipids comprise at least one member selected from the group consisting of phosphatidylcholine, (P C), hydrogenated P C, phosphatidic acid (P A), phosphatidylserine (P S), phosphatidylethanolamine (P E), phosphatidylglycerol (P P G), phosphatidylinositol (P I), hydrogenated P C and similar compounds.
4. A composition according to any of claims 1 to 3, where the alcohol compound is ethanol, and where the mixture of such compounds comprises ethanol and propylene glycol, where the ethanol concentration in the final preparation is between 20 and 50 weight-% of the final product, the content of water being at least about 20 % w/w.
5. A composition according to any of claims 1 to 4, in which the active ingredient is a member selected from the group consisting of peptides, enzymes, hormones, anti-aging agents, tanning agents,

vitamins, antiviral drugs, plant extracts, glycosides, alkaloids, anxiolytics, antiepileptics, antifungals, non-steroidal anti-inflammatory drugs, antihypertensive agents, corticosteroids, minoxidil, cannabinoids, , antibiotics, hydroxy acids, antimitotics, antimycotics, retinoic acid, diclofenac and acyclovir.

6. A composition according to any of claims 1 to 5, where the alcohol is selected from ethanol and isopropyl alcohol, where the glycol is propylene glycol or ethyl diglycol (Transcutol, Gatte fosse, France), or a mixture of any of the alcohols with any of the glycols, or each alcohol by itself.
7. A composition according to any of claims 1 to 6, comprising 22 to 70% of a combination of the alcohol and propylene glycol, and more than 20% water.
8. A process for the production of a cosmetic or medical composition for topical application to the skin, for rapidly introducing into the skin, or for enhanced penetration through the skin, which contains an active ingredient in a carrier defined in any of claims 1 to 7 which comprises mixing a phospholipid, a C₂-to C₄-alcohol, or such alcohol and lower glycol, water, and an active ingredient so as to form a colloid system containing vesicles.
9. A composition according to any of claims 1 to 8, where the alcohol is ethanol, and where the mixture of such compounds comprises ethanol and propylene glycol, where the amount of ethanol is between 20 and 50 weight-% of the composition, the content of water being at least about 20 % w/w.

PL-90 IN WATER
19.200 mg
Rate: 10.0 °C/min
Start temp. -50 °C
End temp. 90 °C
Pan Type 1

File: 00086.001
Ident: 1.0
DSC METTLER
GraphWare TA72

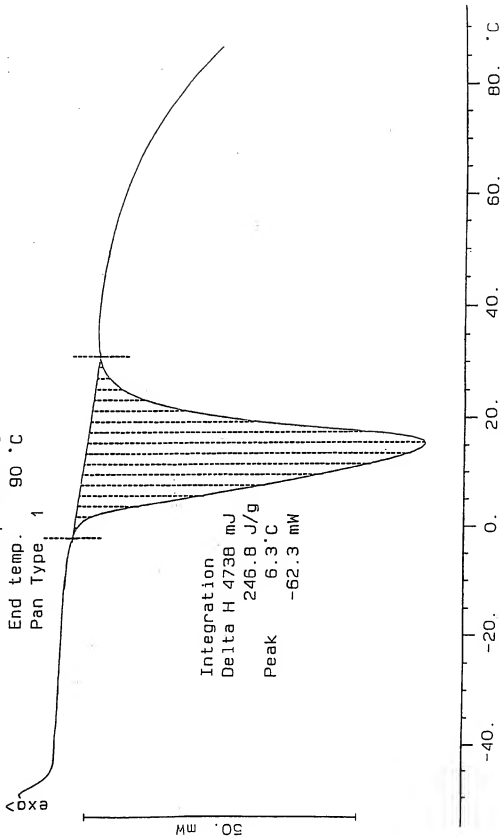


Figure 1: D.S.C of 5% PL-90 in water

#525 PIERCED

22.670 mg

Rate: 10.0 °C/min

Start temp. -50 °C

End temp. 80 °C

Pan Type 1

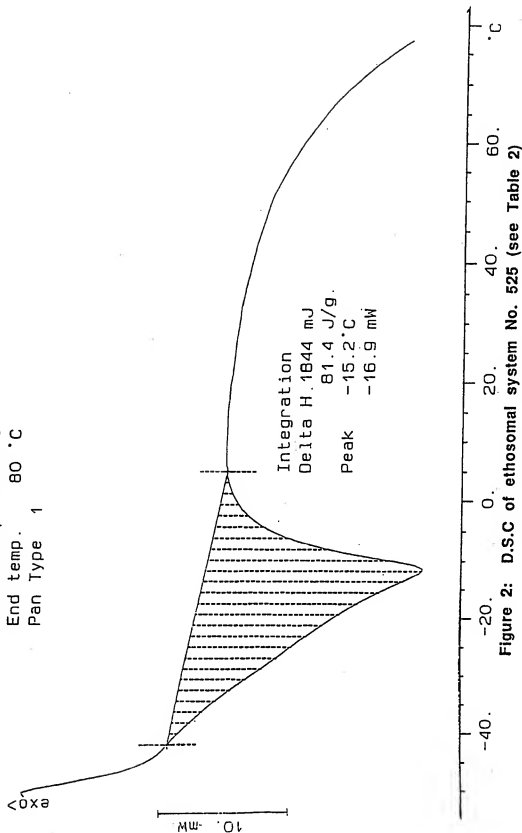
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Ident: 1.0

DSC METTLER

GraphWare TA72

07-Apr-94



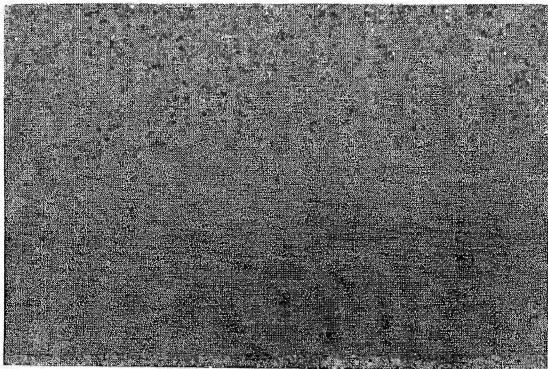


FIG. 3



FIG. 4

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 95/02397

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K9/127 A61K7/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 582 239 (RHONE-POULENC RORER GMBH) 9 February 1994 see page 4, line 5 - line 15 see page 13; example 16 ---	1-9
X	WO,A,91 11993 (NATTERMANN, A&CIE. GMBH) 22 August 1991 see page 13; example 1 see page 16; example 2 see claims 1-8 ---	1-3,5
X	WO,A,92 18103 (PHARES PHARMACEUTICAL HOLLAND B.V.) 29 October 1992 see the whole document ---	1-9
Y	US,A,3 957 971 (OLENIACZ) 18 May 1976 see column 13; example 18 ---	1-9
-/-		

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
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- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another claim or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "A" document member of the same patent family

Date of the actual completion of the international search

21 September 1995

Date of mailing of the international search report

03.10.95

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Benz, K

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 95/02397

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SÖFW- SEIFEN, ÖLE, FETTE, WACHSE, vol. 114, no. 7, 21 April 1988 AUGSBURG (DE), pages 234-237, S. METJE ET AL. 'HERSTELLUNG UND VERMESSUNG VON LIPOSOMEN' see page 236, column 2, paragraph 5 ----	1-9
A	JOURNAL OF PHARMACEUTICAL SCIENCES, vol. 81, no. 2, February 1992 WASHINGTON (US), pages 131-134, XP 000248775 E. TOUITOU ET AL. 'DYPHYLLINE LIPOSOMES FOR DELIVERY TO THE SKIN' see page 131, column 2, paragraph 4 ----	1-9
A	EP,A,0 177 223 (MEZEI) 9 April 1986 see the whole document -----	1-9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 95/02397

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		CN-A- 1084742	06-04-94
		WO-A- 9403156	17-02-94
		PL-A- 302979	05-09-94
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		DE-A- 4003783	14-08-91
		CA-A- 2067807	09-08-91
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		SG-A- 15294	10-06-94
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